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Editor in-Chief's Word

With all joy and anticipation, we celebrate the publication of the second issue of the third volume of the Scientific Journal of the Faculty of Science-Sirte University (SJFSSU). On behalf of the SJFSSU editorial team, I would like to extend a very warm welcome to the readers of SJFSSU. I take this opportunity to thank the authors, editors and anonymous reviewers, who have all volunteered to contribute to the success of the journal. I am also grateful to ***Dr. Al-Senussi Al-Doukali*** and the staff responsible for designing, directing and publishing in our faculty for making SJFSSU a reality.

SJFSSU is dedicated to the rapid publication of high-quality research papers on how advances in science and its applications can help us meet the challenges of the 21st century, and capitalize on the promises ahead. We welcome contributions that can demonstrate practical utility in the near term, especially contributions that take an interdisciplinary approach.

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The SJFSSU was registered in the Arab impact factor database to obtain the factor 1.02 for the year 2022, and it is also indexed in the Marefa database. Moreover, the journal is indexed in the Crossref global platform and has an international Digital Object Identifier (DOI) for the whole journal and for the individual papers.

Finally, we would like to encourage further contributions from the scientific community and industry practitioners to ensure the continued success of the journal. Guest authors, reviewers, and editors are always welcome. We also welcome comments and suggestions that would improve the quality of the journal.

Thank you. We hope you find SJFSSU useful.

Prof. Hamid M. Younis Ahmed

Editor-in-Chief

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Treatment of Turbid Water Collected from Oil Mill Wastewater (OMWW) by Using Low Content of Gum Arabic Polymer (GA)

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A B S T R A C T

Using synthetic polymers in clearyfing of turbid wastewater is very expensive method, in addition to their monomers toxicity produced from the polymerisation reaaction. Those drawbacks encouraged several scientist who intresed in an eco-friendly sustainable materials to discover an upright replacement to clarify turbid wastewater. The present study introduces Gum Arabic (GA) as a health-friendly natural polymer flocculant, which could be applied in pre-filtration process of oil mill wastewaters (OMWW) treatment. GA-cellulose as a model work and GA-OMWW interaction was investigated experimentally by turbidimetric technique and mathematically by GraphPad Prism® software, since plateau followed by one phase decay was good fit at adding 1250 and 12500 mg of polymer, while one phase decay was selected as a proper fit for 12.5 and 125 mg. Results supposed that the biopolymer improves markedly the sedimentation performance of suspended materials. Digital micrographs agreed with the turbidity results.

1 Introduction

Acacia Gum or Gum Arabic (GA) is an exudate produced by the *Leguminosae* tree Acacia Senegal (Fig. 1). It is a complex, branching heteropolysaccharide that can be neutral or slightly acidic and is made up of 1, 3-linked *β*-D-galactopyranosyl units (Fig. 2). This natural macromolecule also contains arabinose, L-rhamnose, and D-glucuronic acid as components (Patel & Goyal, 2015; Shirwaikar et al., 2008). The branches of GA chain are composed of two to five 1, 3-linked *β*-D-galactopyranosyl units, which linkage the main chain by 1, 6-linkages. Some sources report that GA is a mixture of polysaccharides and glycoproteins (Patel & Goyal, 2015). Water dissolves the light-orange or pale-white GA fragments. These plants are found from the west coast of Africa to the Indian peninsula. The majority of GA comes from the arid plains of Sudan, Chad, Nigeria, Senegal, and Ethiopia. Sudan is the greatest exporter, accounting for up to 80% of commerce, and Nigeria is the second largest. The dried saps are extracted as translucent masses, cleaned of foreign matter, kibbled or powdered, and sold to Western countries. GA got its name from Arabian traders who brought it to Europe and helped it become popular (Patel & Goyal, 2015).

Figure (1): Tree with gum Arabic exudates (Musa et al., 2018)

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GA has been used for 5000 years ago (Nasir et al., 2008; Nasir et al., 2012; Patel & Goyal, 2015; Shirwaikar et al., 2008). GA has been used to treat chronic renal disease in Middle Eastern nations (Nasir et al., 2008; Nasir et al., 2012; Patel & Goyal, 2015; Shirwaikar et al., 2008). GA has found widespread use in the food industry due to its edibility, crucial water solubility, generally recognized as safe (GRAS) status, absence of aftertaste, and other favorable properties (Patel & Goyal, 2015).

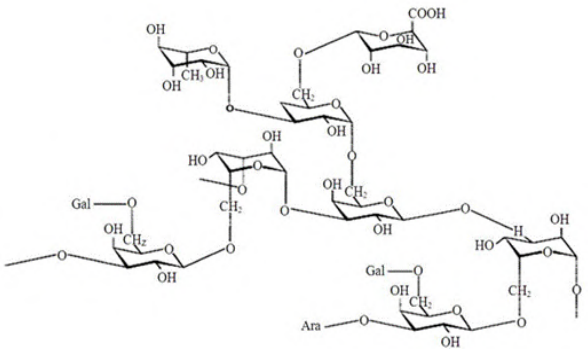


Figure (2): Structure of Gum Arabic.

GA is used in food compositions such as ice creams, jellies, candies, soft drinks, beverages, syrups, and chewing gums due to its emulsifying, stabilizing, thickening, and binding properties. Because of its film-forming characteristics, it is perfect for confectionery coatings and glazes (Patel & Goyal, 2015). Its ability to extend the shelf-life of flavors makes it appealing as a food ingredient (Nasir et al., 2012). The European Union has approved GA for food applications (Shirwaikar et al., 2008). Codex Alimentarius, an international collection of standards, codes of practice, and guidelines, has also endorsed it (Nasir et al., 2008). Its coatings pills and lozenges in pharmaceuticals and natural treatments. It is also utilized in the manufacture of creams and lotions in cosmeceuticals. It is an essential element in traditional lithography, printing, and water color paints due to its great binding property (Patel & Goyal, 2015). GA has also found application in the textile industry due to its potential to improve yarn tensile strength (Nasir et al., 2012). From this perspective, it appears useful to examine the evolution of GA technology over the last decade and the trajectory of future breakthroughs (Shirwaikar et al., 2008).

The quality of GA is determined by factors such as color, odor, moisture and ash content, viscosity, pH, specific rotation, tannins, and metal concentration (Katayama et al., 2006; Nasir et al., 2008; Patel & Goyal, 2015). Ca, Na, K, P, and traces of Pb, Co, Cu, Zn, Ni, Cd, Cr, and Mn are the most common minerals detected. Only when the quality of GA meets international requirements is shipped. As a result, constituent proportions are an important criterion in quality regulation. According to Nasir et al. (Shirwaikar et al., 2008) GA solutions have notably high concentrations of Ca^{2+}, Mg^{2+}, and K^{+}.

The Mediterranean countries that produce olive oil, such as Spain, Italy, Greece, and Tunisia, are confronted with the problem of removing the agro-industrial residues that result from this production, namely, olive-oil mill wastewaters (OMWWs), which account for approximately 1-1.6 m^3 per ton of olive fruits processed. The worldwide estimated volume of OMWW produced during the olive oil extraction process is 7-30 million m^3 per year, with Mediterranean areas producing 30 hm^3. This affluent has a high saline charge and is acidic, with a brown aqueous liquid with a hazy appearance. Its heavy organic load of sugars, tannins, polyalcohols, pectins, lipids, and primarily phenolic chemicals renders it practically unbiodegradable by ordinary biological processes (Hachicha et al., 2023). Olive oil mill wastewater (OMWW) has recently expanded dramatically due to the rapidly expanding demand for olive oil and current oil extraction processes that require large amounts of water. (Benitez et al., 1997; Lourenço et al., 2017).

The composition of the generated effluent varies according to climate and milling procedures (Tsigkou et al., 2022). OMWW has properties such as a high concentration of particles due to washing operations, a deep dark hue, an acidic pH, and a pungent odor (Tsigkou et al., 2022). When these wastes are disposed of in the environment, they produce major difficulties such as water coloring and pollution, changes in soil quality, plant growth suppression, and odor nuisance (Marques, 2001). Furthermore, direct runoff on fields reduces dissolved oxygen levels, hurting aquatic biodiversity (Marques et al., 1998). As a result, OMWW must be treated before disposal, and different treatment technologies must be combined to create an acceptable and effective means of dealing with the produced wastewater (Hamdi & Ellouz, 1992).

There are numerous strategies for treating OMWW; biological treatment, co-digestion, aerobic and anaerobic digestion are some examples (Hamdi & Ellouz, 1992). Nonetheless, the OMWW contains a high concentration of fats, lipids, and phenols, which can inhibit the growth of microbes (Cereti et al., 2004). Co-digestion is the co-treatment of one wastewater with another, which has the advantage of supplying the required pH or nutrient level for further treatment (Borja et al., 1995; Borja et al., 1995). The aerobic treatment stage can lower toxicity by reducing phenols (Borja et al., 1995; Borja et al., 1995).

This work aims to use gum Arabic (GA) as a health-friendly polymer that could be introduced as a good replacement instead of synthetic polymers in clarification of turbid water collected oil mill waste (OMWW) using low content of Gum Arabic polymer.

2 Materials and Methods

All materials in the present study have been used without further purifications unless was mentioned.

2.1 Materials

In this work GA from soluble pure, as shown in Fig. 1 was obtained from a local store. Cellulose (Sigma). Double distilled water (H_2O) (Research laboratory). Sodium Azide (NaN_3) (Sigma). Propanol (C_3H_8O) (Sigma). Olive oil mill waste (Local oil mill) in Benghazi city (Libya).

2.2 Synthetic Turbid Water: Model Work

Synthetic turbid water was prepared by dispersion of 10 grams of cellulose as a model suspended material, in 100 mL of water, immediately turbidity was recorded as a function of time, the same protocol was repeated but by adding 10, 100, and 1000 mg of GA respectively. Turbidity was measured by a conventional turbidity meter (2020 Nephelometer), after calibration with distilled water.

2.3 Kinetic Experiment

12.5, 125, 1250, and 12500 mg of GA polymer were added to 250 mL of OMWW water in separated beakers, then the turbidity was measured immediately versus time in minutes scale. For kinetic study data were fitted to an exponential function via Graph Pad Prism Software®. The final turbidity was also measured after filtration after each addition of GA polymer.

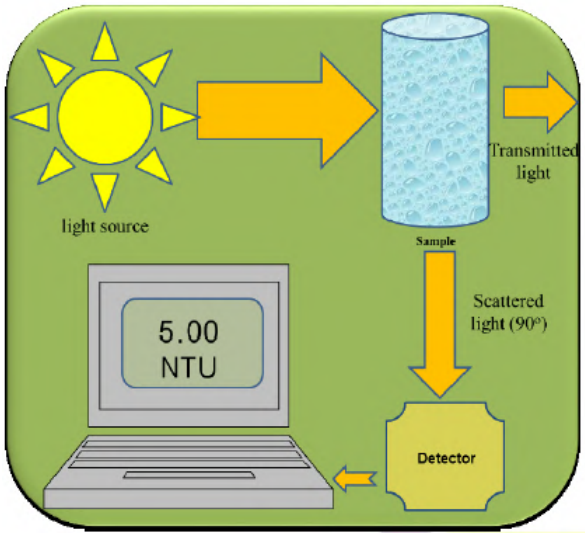


Figure (3): Turbidity measurements by 2020 Nephelometer.

2.4 Statistical Analysis

Results were expressed as means ± standard division of the mean (n = 3).

3 Results

The interaction of a polymer with suspended materials is accompanied by changes in the turbidity of the mixture solution (Eltaboni et al., 2015). Since the adhesion of this macromolecule onto solids led to settling down of the polymer chains, as a result, the suspension concentration decreases in the bulk solution, thus the turbidity of the supernatant solution will be decreased (Eltaboni et al., 2015). A versatile technique like a turbidity meter, which monitors polymeric solution transmittance, should be useful for investigating macromolecule-solids sedimentation.

3.1 Model Study of Gum Arabic-Cellulose Interaction

In order to prove the ability of gum Arabic (GA) polymer for acting as a good natural sedimentation agent, a model study was designed by monitoring the decay in the turbidity values as a function of GA-cellulose interaction time at different amounts of polymer, as shown in Fig 4. As an overall trend, the addition of polymer to the cellulose suspension exhibited a marked decay in turbidity over a stated period of time (2 hours), after this time no significant change was observed. It's worthy to note that the turbidity decay at 1000 mg of GA addition is lower than that of 10 and 100 mg of added GA, since at which turbidity decreased from about 60 NTU to 20 NTU.

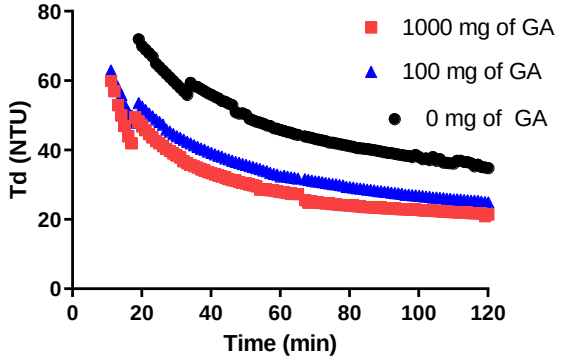


Figure (4): Turbidity decay of 10 wt% of cellulose suspension in different Gum Arabic additions (10, 100, and 1000 mg) at pH 7 and 25 °C.

3.2 Effect of Gum Arabic Content on the OMW Water Turbidity

Despite the OMWW being a turbid suspension, its turbidity varied over time from approximately 100 NTU to 65 NTU, as depicted in Fig 5. The final decayed

turbidity was still high compared with the turbidity of distilled water which is less than 5 NTU as stated by Ministry of Health standard (Azman et al., 2016).

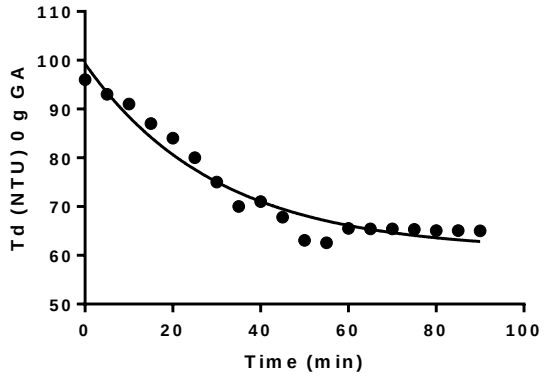


Figure (5): Turbidity of OMWW suspension as a function of time (without adding GA).

The mathematical fitting by Prism software for the experimental turbidity results in Fig 5 was carried out via using one phase model (equation 1), the generated lifetime of sedimentation for OMWW suspension and its related parameters are displayed in Table 1.

$$Td = (Td_0 - Plateau)*\exp^{(-K*t)} + Plateau \quad (1)$$

Where Td_0 is the turbidity (Td) value when t (time) is zero. It is expressed in the same units as Td (NTU), Plateau is the Td value at infinite times, expressed in the same units as Td. K is the rate constant, expressed in the reciprocal of the time in minutes. τ is the time constant, expressed in the same units as the t. It is computed as the reciprocal of K. Half-life ($t_{0.5}$) is in the time at which the half amount of OMWW is stilled down on the bottom of the container. It is computed as ln (2)/K. All the fitted data is statistically acceptable with a good regression coefficient $R^2 = 0.9507$.

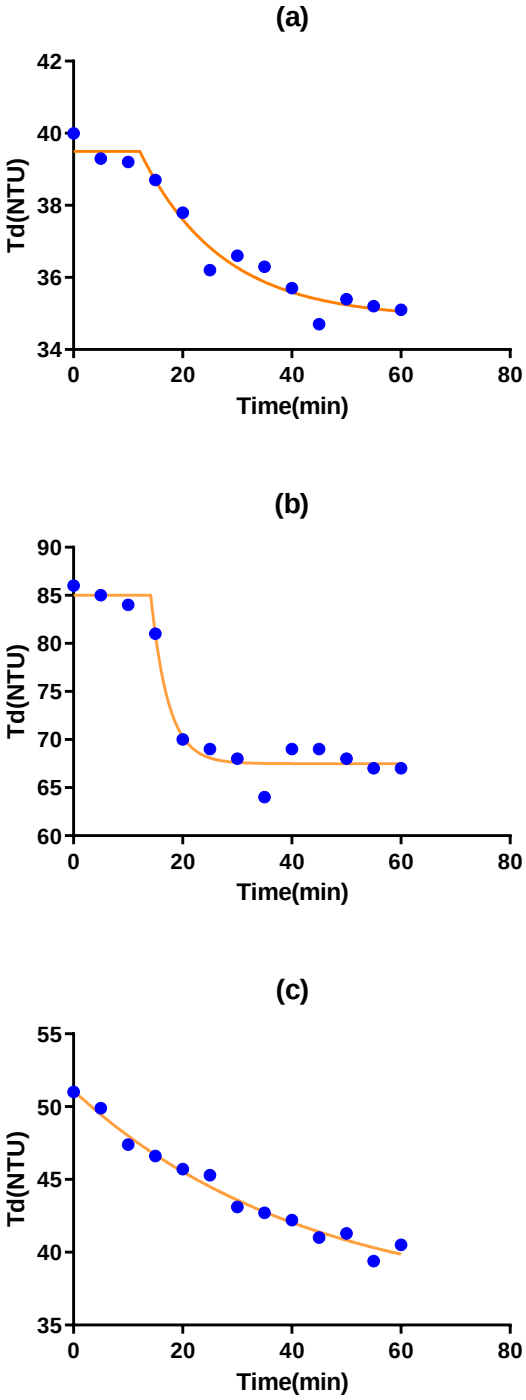
Table (1): OMWW suspension lifetime and its parameters.

One phase decay parameter	Value
K (min^{-1})	0.03329
$t_{0.5}$ (min)	20.82
τ (min)	30.04
R^2	0.9507

3.3 Kinetic study of gum Arabic interaction with OMW water

As proved by the model study, the effectiveness of GA polymer as a natural coagulant could make this naturally occurring material a credible alternative to current synthetic polymers. The kinetic study of GA-OMWW interaction was carried out by monitoring the decrease in the turbidity values as a function of time in minutes units, as shown in Fig 6 (a, b, c, and d), the dots represent the real data and the solid line decay represent the mathematical fitting. Fig 6 (a) and (b) plateau followed one phase least squares fit was found suitable to simulate the experimental results (equation 2).

$$Td = IF(t<t_0, Td_0, Plateau+(Td_0-Plateau)*\exp^{(-K*(t-t0))}) \quad (2)$$



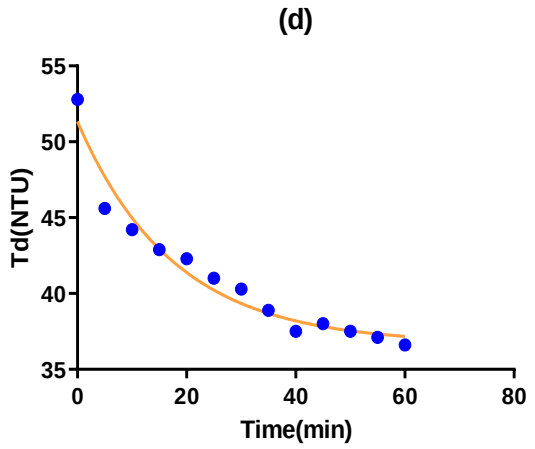


Figure (6): Kinetic decay of OMWW-GA interaction at 25 ºC an pH 7, adding 12.5 mg (a), 125 mg (b), 1250 mg (c), and 12500 mg of GA polymer.

Unlike the Fig 6 (a and b), in Fig 6 (c and d) the mathmatical fitting for the exprimental data was done by using one phase least sequares fit (equation 1), the produced lifetime of that interaction and its related parameters are dipicted in Table 2.

Table 1. Kinetic data for OMWW-GA interaction.

Kinetic parameter	12.5 mg of GA	125 mg of GA	1250 mg of GA	12500 mg of GA
K (min^{-1})	0.06675	0.3124	0.02342	0.05688
$t_{0.5}$ (min)	10.38	2.219	29.60	12.19
τ (min)	14.98	3.201	42.70	17.58
R^2	0.9599	0.9726	0.9806	0.9578

As shown in Table 2, the rate constant of GA-OMWW interaction had its maximum value when 125 mg of the polymer was added, while the lower value of K was recorded during adding 1250 mg of GA. In contrast, the half and lifetimes of interaction reached the maxima at the addition of 1250 mg of polymer. But only 3 minutes were enough to get an interaction between the suspended materials and the polymer.

Fig 7 shows the turbidity dependence on added polymer, which was measured after filtration to remove the precipitated GA-OMWW. As an overall trend, increasing the amount of added polymer was accompanied by a slight increase in the turbidity of the bulk solution.

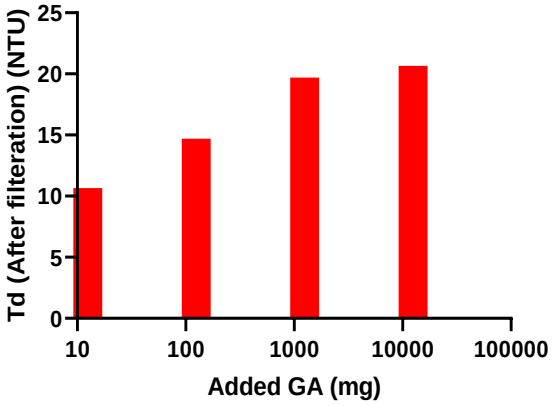


Figure (7): Turbidity of bulk solution as a function of GA amount after filteration (at natural pH and room temperature)

3.4 Digital Micrographs of OMW Water Samples

As it can be observed from the turbidity results, when the GA polymer was added to the cloudy OMWW suspension a clear water was formed. To support that results digital micrographs were taken for OMWW treated with GA as shown in Figure 8.

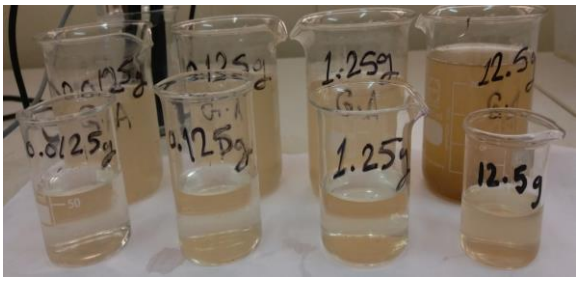


Figure (8): Clreafication of OMWW by adding different amount of GA polymer.

4 Discussion

Fig 4 shows the kinetic study of GA interaction with a cellulose suspension, as a model work, the experiment was determined by monitoring the variation in turbidity values as a function of time in minutes scale and was done at room temperature in natural pH medium. As it can be noted that without adding GA polymer the value Td decreased up to 40 (NTU) but by adding GA the value decreased to about 20 (NTU), this could be because of a quick interaction (occurred in 30 minutes, Table 1) occurred between GA chain and cellulose molecules, as proposed in Fig 9, the result of this binding let the suspended cellulose to settle down in the bottom of the aqueous solution, as a result, a clear solution (decrease in turbidity) is noted. This study proved that model-suspended cellulose was a good candidate to examine the

ability of GA to eliminate any suspensions that could exist in the OMWW sample. The suggested mechanism is in good agreement with our previous studies which used the fluorescence technique to monitor silica binding with polymers similar in structure to GA like polyacrylic acid, alginate, and lipopolysaccharides (Sparks et al., 2015; Eltaboni et al., 2020; Eltaboni et al., 2022).

Table 1 displays lifetime (τ) and its parameters for OMWW suspension. The lifetime was determined using the turbidity technique. A one phase decay model (Equation 1) was used to fit the kinetic data, hence the stability of the suspension lifetime was simulated. The results in Table 1 show that the first order model is a good fit for experimental data as its corresponding R^2 value is considerably high (0.9507). The turbidity values of suspension were found to be time dependent. It was found that the turbidity decays until reaching a plateau with the lifetime reached to 30 minutes, combined with a rate constant equals 3.3 x 10^{-2} min^{-1}.

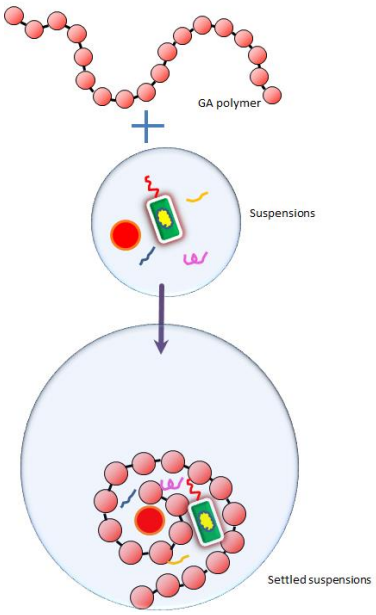
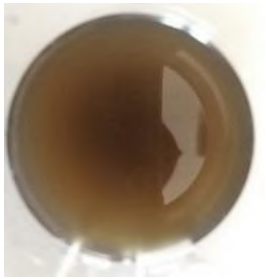


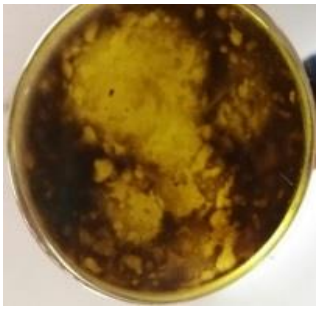
Figure (9): Proposed scheme shown cloudy water suspended with different materials turned clear when GA was added.

When OMWW samples were treated with 12.5, 125, 1250 and 12500 mg of GA (Fig 6 a-d), it was interestingly found that at low content of gum Arabic the polymer-OMWW particles remain suspended in solution for a couple of minutes and then stilled down on the bottom as a result of both particle interaction. This suggestion was confirmed by plateau following one phase model with a good regression coefficient (Table 2). On the contrary, at adding 1250 and 12500 mg of GA a direct interaction happened between polymer and OMWW, which was confirmed by one phase decay. However, increasing the amount of polymer could let some of it undissolved in solution and this was accompanied by an increase in the turbidity values (Fig 7).

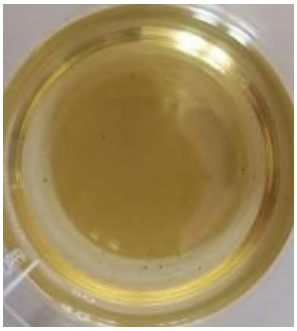
The proposed mechanism in Fig 9 was established by Fig 10, in which the variation in clarification of OMWW suspension upon adding polymer proposed that a kind of interaction occurred between suspended materials and GA.



(a)



(b)



(c)

Figure (10): Initial OMWW (a) and OMWW after treatment with GA flocculant(b), and filtrate (c), in optimized conditions of pH and concentration.

5 Conclusion

The experimental results showed that the highest efficiency for GA as water clearer was increased by increasing its content to 12500 mg per 250 mL of treated

OMWW samples, before filtration. Possibly, the increase in the amount of settling suspension upon adding polymer causes a decrease in the turbidity. Since the stronger interaction between the GA-OMWW is more favorable. Digital micrographs agreed with the turbidity results, these proposed that health friendly polymer (GA) could be a promising material to use in OMW treatment, but further examination should be carried out to get pure water.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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Chemical Screening, antioxidant activity, and Mineral Profiling of *Rosmarinus Officinalis* L. from Msallata Region (Libya)

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A B S T R A C T

This study aimed to investigate the phytochemical constituents, total phenols, total antioxidant, and mineral contents of *Rosmarinus Officinalis* L. The aerial parts of the plant were extracted using different solvents, including water, ethanol, ethyl acetate, and chloroform. The extracts were analyzed for their phytochemical constituents. The total phenolic content and total antioxidant capacity were measured using colorimetric assays. The mineral content of the plant was analyzed using AAS. The results showed that the plant extracts were rich in carbohydrates, proteins, phenols, alkaloids, flavonoids, tannins, glycosides, and coumarins. The total phenolic content of the plant extracts ranged from 76.7 to 95.5 mg GAE/g, indicating strong antioxidant activity, with a total antioxidant capacity of 50.03 mg/g. The plant was found to be rich in macro elements such as Na, K, Ca, Mg, and P, with concentrations ranging from 1760 to 12155 mg/kg. While the concentrations of heavy metals (Fe, Zn, Cu) ranged from 56.19 mg/kg to 14.38 mg/kg. The water extract contained fewer phytochemical constituents compared to the ethanolic extract, while the ethyl acetate and chloroform extracts showed the presence of only specific compounds. These findings suggest that *Rosmarinus Officinalis* L. is a valuable natural source of bioactive compounds and essential nutrients. Further research is needed to fully elucidate the therapeutic properties of the plant and to explore its potential use in the prevention and treatment of various diseases.

1 Introduction

Throughout history, herbs and plants have been utilized for a multitude of purposes, including medicine, pharmaceuticals, nutrition, food preservation, flavorings, beverages, repellents, fragrances, and cosmetics (Sarker & Nahar, 2022). They were the basis for medicinal therapy until the development of synthetic drugs in the nineteenth century (Verma *et al.*, 2021). In recent decades, there has been a renewed interest in the use of herbs and plants due to their natural bioactive compounds, such as polyphenols, vitamins, polysaccharides, and minerals, which offer various beneficial activities (Elsherif *et al.*, 2023; Costa *et al.*, 2021). With their mild features and low side effects, the use of natural compounds is increasing worldwide. Herbal-based cosmetic preparations have also become popular among consumers due to their non-toxic nature and strong activity (Javed & Shoaib, 2021).

Rosemary, scientifically known as *Rosmarinus officinalis* L., is a fragrant and perennial plant that belongs to the Lamiaceae family and originates from the Mediterranean region, but it is now widely distributed worldwide. This shrub-like plant can grow up to two meters high and is characterized by its green leaves that emit a pleasant aroma. Rosemary is versatile and can be used as a spice in cooking, a natural preservative in the food industry, and as an ornamental and medicinal plant (Silva & Dias, 2022; Khalil *et. al*, 2021). Rosemary (*Rosmarinus officinalis*) has been traditionally used for its medicinal properties, including antispasmodic, diuretic, antirheumatic, and antiepileptic effects.

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Moreover, it is effective in treating respiratory problems, skin infections, and promoting wound healing (Al-Snafi, 2021) and (Mocan *et al.*, 2021). Rosemary (*Rosmarinus officinalis*) contains various bioactive compounds, such as flavones, diterpenes, steroids, and triterpenes. Among these compounds, the antioxidant activity of rosemary extracts has been mainly attributed to two phenolic diterpenes, carnosic acid and carnosol (Silva & Dias, 2022; González-Trujano *et al*., 2021; Naveed *et al*., 2021). The chemical composition and biological activities of rosemary has attracted the attention of many research works. (Silva & Dias, 2022) conducted a comprehensive review of *Rosmarinus officinalis* L., highlighting its chemical composition, biological activities, and applications in food preservation. The authors (Silva & Dias, 2022) reported that rosemary contains a high level of antioxidants, particularly phenolic compounds such as carnosic acid and carnosol, which contribute to its potent antioxidant activity. Total phenolic content and mineral determinations of rosemary extracts were also found to vary depending on the extraction method and plant part used. In addition, phytochemical screening of rosemary revealed the presence of various bioactive compounds, including flavonoids, triterpenes, and steroids (Sakar *et al.*, 2021). The review also discussed the potential uses of rosemary in food preservation due to its natural antimicrobial properties, which can inhibit the growth of foodborne pathogens and extend the shelf life of food products. Overall, the findings suggest that rosemary is a promising natural source of antioxidants and could be utilized in various applications, including food, pharmaceuticals, and cosmetics (Silva & Dias, 2022).

This study aimed to identify the bioactive compounds present in four *Rosmarinus officinalis* L. extracts (water, ethanol, ethyl acetate, and chloroform) through qualitative phytochemical screening. Additionally, the study sought to determine the moisture and ash contents, as well as the levels of total phenols and antioxidants in the plant. The concentrations of potassium, sodium, calcium, magnesium, phosphorus, copper, iron, and zinc in the herbal plant sample were also measured using flame photometry and flame atomic absorption spectrometry.

2 Materials and Methods

2.1 Study Area

The *Rosmarinus Officinalis* leaves were harvested from the Msallata region of Libya.

2.2 Chemicals and Equipment

The study utilized solvents such as water, ethanol, chloroform, and ethyl acetate for extraction, as well as various reference materials and reagents including ascorbic acid, gallic acid, sodium phosphate, ammonium molybdate, and Folin–Ciocalteu reagent, which were all obtained from the Sigma-Aldrich distributor. All the chemicals, reagents, and solvents used in the study were of analytical grade unless otherwise specified. In terms of equipment, the study employed a rotary evaporator (Hei-VAP, Heidolph, Schwabach, Germany), a UV-Vis spectrophotometer (6300, Jenway), an Atomic Absorption System (AAS) (Varian 220 FS), and a Flame Photometer (PFP7, Jenway) for analysis purposes.

2.3 Sample Collection

The *Rosmarinus Officinalis* leaves were harvested from the Msallata region of Libya, during January, February, and March 2021. To ensure their authenticity, the leaves were authenticated by a botanist at the Faculty of Arts and Science, Elmergib University, Alkhoms, Libya.

2.4 Preparation of Plant Sample

To prepare the plant samples for analysis, they were thoroughly washed with tap water followed by distilled water to remove any dirt and dust. After washing, the plant samples were air-dried at room temperature for a period of 25 days to ensure complete drying. Once dried, the samples were ground using an electric grinder, sieved to obtain a uniform particle size, and stored in sealed glass bottles to prevent any moisture or contamination.

This process ensured that the plant samples were properly prepared for the subsequent analyses.

2.5 Extraction Method

The plant powder was extracted with 200 mL of solvent (distilled water, ethanol, chloroform, or ethyl acetate) using a 20 g/200 mL ratio, following the method described in literature (Harborne, 1973; Elsherif & Aljaroushi, 2021a). The mixture was allowed to stand for 72 hours at room temperature, before being filtered with Whatman No.1 filter paper. The resulting filtrate was then concentrated using a water bath and dried in an oven at 40°C to obtain a brownish-black semi-solid extract.

The crude plant extracts were collected and stored in airtight glass bottles in the refrigerator for further use. These extracts were utilized for performing phytochemical screening and evaluating the total phenols and total antioxidant activity of the plants.

2.6 Yield

The extraction yield, which represents the amount of dry extract obtained per 100 g of fresh plant sample, was determined. To calculate the extraction yield, the solvent-extracted plant was evaporated in a water bath at 40°C and subsequently dried for 24 hours in an air oven at 40°C. The extraction yield was then calculated based on the final dry weight of the extract using the following equation (Najah & Elsherif, 2016):

$$\% \text{ Yield} = \frac{\text{Wt of dry extract (g)}}{\text{Wt of fresh sample (g)}} \text{ X } 100 \qquad (1)$$

2.7 Phytochemical Screening

In the present study, several compounds including carbohydrates, proteins, phenols, alkaloids, flavonoids, tannins, saponins, steroids, glycosides, coumarins, and terpenes were identified in the four plant extracts (aqueous, ethanolic, ethyl acetate, and chloroform). The identification of these compounds was carried out using established procedures found in the literature (Sofowora, 2008; Najah *et al.*, 2015).

2.8 Moisture and Ash Contents

The moisture and ash content of the plant sample were determined using the Association of Official Analytical Chemists' official methods of analysis (AOAC, 2005; Elsherif & Aljaroushi, 2021b). These methods involve measuring the mass of water in a known amount of sample before and after evaporation. The moisture content was calculated using the following equation:

$$\% \text{ Moisture} = \frac{wt_1 - wt_2}{wt_1} \text{ X } 100 \qquad (2)$$

Where; wt_1: weight (g) of plant sample before drying and wt_2: weight (g) of plant sample after drying. To determine the moisture content of a plant sample using evaporation methods, the sample is first dried in an oven at a specific temperature (e.g., 60°C) for a set period of time (e.g., 1 hour). Once removed from the oven, the sample is allowed to cool in a desiccator and is then weighed in grams. This process is repeated several times until a constant weight is obtained, and the moisture content is calculated using the initial and final weights (wt_1 and wt_2) of the sample. It is crucial to remove all the water molecules that were initially present in the plant without changing the mass of the plant matrix to obtain an accurate measurement of the moisture content. However, it is important to note that factors such as temperature, humidity, and duration of drying can impact the accuracy of the results obtained through this method.

The ash content of the plant samples was determined using a method described in the literature (AOAC, 2005; Elsherif & Aljaroushi, 2021a). Initially, a gram of plant material was dried in an oven at a temperature range of 100°C to 105°C. The dried sample was then ashed in a muffle furnace for one and a half hours, gradually increasing the temperature from 100°C to 600°C. The ash was then placed in a desiccator to cool down, weighed, and the ash content was calculated using equation (3):

$$\% \text{ Ash} = \frac{\text{Wt of ash (g)}}{\text{Wt of fresh sample (g)}} \text{ X } 100 \qquad (3)$$

It's worth noting that the ash content of a sample can be influenced by several factors, including the type of plant material, the temperature and duration of ashing, and the presence of impurities (Ahmed *et al.*, 2016).

2.9 Estimation of Total Phenolic Contents

The total phenolic content of the plant extracts was determined using a slightly modified version of the Folin-Ciocalteu reagent method (Kumar & Sagrawat, 2021). This method involves the conversion of phenols to phosphomolybdate-phosphotungstic acid in an alkaline medium, resulting in a blue-colored solution whose absorption at 760 nm is measured.

To perform the assay, 0.25 ml of the ethanolic extract was mixed with 1.0 ml of dilute (1:10) Folin-Ciocalteu reagent in a test tube, which was then diluted to 10 ml with distilled water. After 5 minutes in the dark, 0.8 ml of 7.5% Na_2CO_3 solution was added to each tube, and the solution was thoroughly mixed by hand. The test tubes were then kept in a dark place for 60 minutes before measuring the absorbance of the samples with a UV spectrophotometer at a fixed wavelength of 760 nm.

The concentration of phenolic compounds in the extracts was evaluated using a gallic acid calibration curve. Gallic acid standards were prepared at concentrations of 10, 20, 40, 60, 80, and 100 mg/L to create the calibration curve. The amount of phenolic compounds in the extracts was expressed as gallic acid equivalence (mg gallic acid/g dry sample).

It is important to note that the Folin-Ciocalteu reagent method is a widely used assay for the determination of total phenolic content in plant extracts, although it may not be specific for all types of phenolic compounds (Singleton *et al.*, 1999).

2.10 Determination of Total Antioxidants Activity

The total antioxidant capacity (TAC) of the ethanolic extract of *Rosmarinus Officinalis* was determined using the phosphomolybdenum assay, as described in the literature (Yılmaz *et al.*, 2021). The assay involves the reduction of Mo(VI) to Mo(V) by the plant extract's antioxidant compounds, resulting in the formation of a green phosphate/Mo(V) complex. The complex's absorbance is measured spectrophotometrically at 695 nm, and the TAC is expressed in milligrams of ascorbic acid equivalents (mg ascorbic acid/g dry sample).

To perform the assay, 0.3 mL of each extract solution in ethanol was mixed with 3.0 mL of phosphomolybdenum reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulfuric acid) in capped test tubes. The samples were then incubated for 60 minutes in a 95°C water bath. After cooling to room temperature, the absorbance of the solutions was measured against a blank using a UV-visible spectrophotometer at 695 nm (0.3 mL ethanol without plant extract).

To calculate the TAC of the extracts, an ascorbic acid calibration curve was used. Ascorbic acid standards were prepared at concentrations of 20, 40, 60, 80, 100, and 120 mg/L. The TAC values were expressed in mg of ascorbic acid equivalents (mg ascorbic acid/g dry sample).

It is important to note that the phosphomolybdenum assay is a commonly used method for the determination of TAC in plant extracts. However, this assay may not be specific for all types of antioxidants (Apak *et al.*, 2020).

2.11 Metal Contents

The mineral and heavy metal content of the powdered samples was determined using a Jenway flame photometer and a VARIAN 220 atomic absorption spectrometer to measure the concentrations of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), iron (Fe), and zinc (Zn) (Elsherif & Kuss, 2012; Elbagermi *et al.*, 2020). The samples were prepared for analysis using the dry digestion method (Noubigh & Oubrim, 2021; Alkherraz *et al.*, 2019).

To prepare the sample, a porcelain crucible was used to hold 1.0 g of the material. The furnace temperature was gradually increased from room temperature to 550°C over a period of one hour. The sample was then ashed for approximately 8 hours until a white or grey ash residue was obtained. To dissolve the residue, it was mixed with 2.0 ml of concentrated HNO_3 and heated slowly as needed. The resulting solution was transferred to a 100-ml volumetric flask and brought up to volume. A blank control was prepared in the same manner using only the solvent. The samples were stored in polyethylene containers in a refrigerator until analysis.

Prior to analysis, all glassware was cleaned by soaking in a 10% nitric acid solution overnight and rinsing three times with distilled water. It is worth noting that the determination of mineral and heavy metal content in samples is essential for assessing their nutritional and toxicological properties (Moshtaghian & Mohammadi, 2022). However, the accuracy of the results may be affected by various factors, such as the type of sample, the analytical method used, and the sample preparation procedure (Dabrowska & Zielinski, 2021).

3 Results and Discussion

3.1 Phytochemical Screening

The *Rosmarinus Officinalis* L. plant extracts (ethanol, aqueous, chloroform, and ethyl acetate) were subjected to phytochemical screening experiments which revealed the presence of various bioactive compounds with known medicinal significance. The findings of the phytochemical screening are presented in Table 1. It is important to note that the detection of these compounds in the extracts suggests their potential use in therapeutic applications.

The phytochemical screening of *Rosmarinus Officinalis* L extracts in ethanol, aqueous, chloroform, and ethyl acetate revealed the presence of various bioactive compounds. The ethanol extract tested positive for carbohydrates, proteins, phenols, alkaloids, flavonoids, tannins, glycosides, and coumarins, but did not contain saponins or steroids. Similarly, the water extract contained carbohydrates, proteins, phenols, alkaloids, tannins, and saponins, but lacked flavonoids, steroids, glycosides, and coumarins. The ethyl acetate extract, on the other hand, only showed the presence of carbohydrates, alkaloids, flavonoids, saponins, and coumarins, whereas the other compounds were not detected. The chloroform extract, in turn, tested positive only for proteins, steroids, and flavonoids, while the remaining compounds were absent.

Comparing these results with the literature on *Rosmarinus Officinalis* L, previous studies have reported the presence of various phytochemicals in the plant, including phenolic compounds, flavonoids, alkaloids, tannins, and terpenoids (Zengin *et al.*, 2013). These compounds have been shown to possess a wide range of pharmacological properties, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (Ben Jemaa *et al.*, 2019). The presence of these compounds in the extracts of *Rosmarinus Officinalis* L suggests its potential use in the development of natural remedies for various health conditions.

Table 1: Phytochemical screening of four extracts of *Rosmarinus Officinalis* L.

Test		Solvents			
No	detection test	ethanol	water	ethyl acetate	chloroform
1	Steroids and triterpenes (Lieberman)	-	-	-	+
2	Coumarins (NaOH)	+	-	+	-
3	Flavonoids (NaOH)	+	-	+	+
4	Alkaloids (Wagner)	+	+	+	-
7	Tannins (lead acetate)	+	+	-	-
8	Phenols (ferric chloride)	+	+	-	-
11	Carbohydrates (Molisch)	+	+	+	-
12	turbines	-	-	-	-
13	saponins	-	+	+	-
14	Glycosides	+	-	-	-
15	Proteins	+	+	-	+

- (+): present; (−): absent

3.2 Yield

The yields of *Rosmarinus Officinalis* L extracts in ethanolic, aqueous, ethyl acetate, and chloroform (as shown in Table 2) were found to be 13.95%, 12.35%, 8.93%, and 10.75%, respectively. These results indicate that the highest yield was obtained from the ethanolic extract, followed by the aqueous, chloroform, and ethyl acetate extracts. The yield of the extracts is an important

factor to consider in the development of natural remedies, as it affects the quantity and quality of the bioactive compounds present in the extract. Therefore, the yield of *Rosmarinus Officinalis* L extracts could have an impact on their potential therapeutic applications. The results are depicted also in Figure 1.

Comparing these results with the literature on *Rosmarinus Officinalis* L, previous studies have reported varying yields for the plant's extracts. For example, one study reported a yield of 8.5% for the ethanolic extract (Tavakkoli *et al.*, 2017), while another study reported a yield of 13.4% for the aqueous extract (López *et al.*, 2006). In contrast, a third study reported a yield of 2.8% for the ethyl acetate extract (Ali *et al.*, 2016). The differences in the yield of the extracts could be attributed to several factors, including the plant part used, the extraction method, and the solvent used. Nevertheless, these studies highlight the variability in the yield of *Rosmarinus Officinalis* L extracts and suggest the need for standardization in the extraction process to ensure consistent yield and quality of the extracts.

Table (2): Results of yield, moisture, and ash contents of *Rosmarinus Officinalis* L

%	Ethanolic extract	Aqueous extract	Ethyl acetate extract	Chloroform
Yield	13.95	12.35	8.93	10.75
Moisture	14.00			
Ash	10.00			

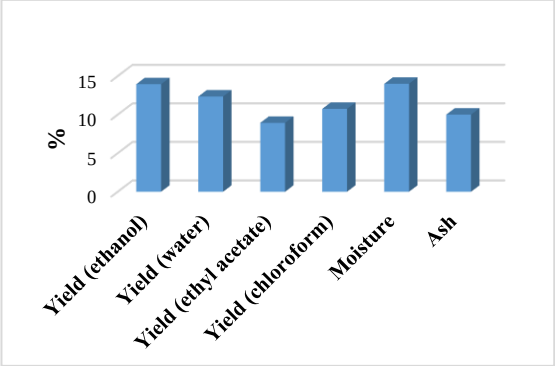

Figure (1): The Yield, moisture, and Ash contents of the studied plant

3.3 Moisture and Ash Contents

Table 2 presents the results of the moisture and ash content analysis of the studied plant, *Rosmarinus Officinalis* L. The ash content was found to be 10.00%, while the moisture content was 14.00%. These findings are important in determining the quality and purity of the plant material. The ash content reflects the inorganic residue left after the plant material is burned, which can indicate the presence of impurities or adulterants. On the other hand, the moisture content is an important factor to consider in the storage and preservation of the plant material, as excessive moisture can lead to spoilage and degradation of the plant material (Gupta & Sharma, 2006).

Previous studies have reported similar results for the plant's ash and moisture content. (Jamshidi-Kia *et al.*, 2018) reported an ash content of 9.8% and a moisture content of 11.2% for the aerial parts of the plant. Similarly, (Górnicki & Kasprzykowski, 2012), the study reported an ash content of 10.7% and a moisture content of 13.2% for the plant's leaves. These findings suggest that the ash and moisture content of *Rosmarinus Officinalis* L may be consistent across different studies, which could support the standardization of the plant material for its use in natural product research.

3.4 Total Phenols Content

The phenolic content of ethanolic extract was determined using the Folin–Ciocalteu reagent and expressed in gallic acid equivalents (GAE) per gram dry extract weight, based on a calibration curve ($y=0.0058x$, $R^2=0.9704$) of gallic acid in the concentration range of 10–80 mg/L (Figure 2). The ethanolic extract exhibited a content of phenolic compounds of 76.7 ± 4.84 mg GAE/g (Figure 3). Figure 4 illustrates a positive correlation between the total phenolic content and extract concentration, indicating that the higher the concentration of the extract, the higher the phenolic content. These results suggest that the ethanolic extract of *Rosmarinus Officinalis* L may be a potential source of phenolic compounds with possible health benefits.

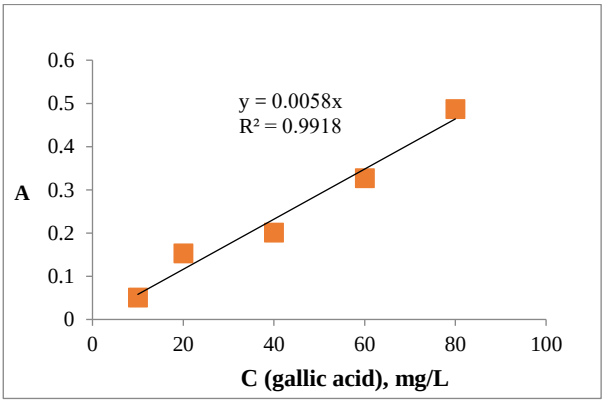

Figure (2): Calibration curve of gallic acid

It is worth noting that the Folin–Ciocalteu method is a widely used assay for the determination of total phenolic content in plant extracts. Previous studies have also used this method to quantify the phenolic content of *Rosmarinus Officinalis* L extracts. For instance, (Ali *et al.*, 2016) reported a total phenolic content of 44.89 mg GAE/g in the ethanolic extract of *Rosmarinus Officinalis* L, which is consistent with the findings of the present study. Another study by (López *et al.*, 2006) reported a total phenolic content of 35.8 mg GAE/g in the aqueous extract of *Rosmarinus Officinalis* L. (Hosseini *et al.*, 2010) study found that the total phenolic content of

Rosmarinus Officinalis L extract was 46.7 mg GAE/g, which is consistent with the results obtained in this study for the ethanolic extract. These studies suggest that *Rosmarinus Officinalis* L extracts are a rich source of phenolic compounds, which could have potential health benefits.

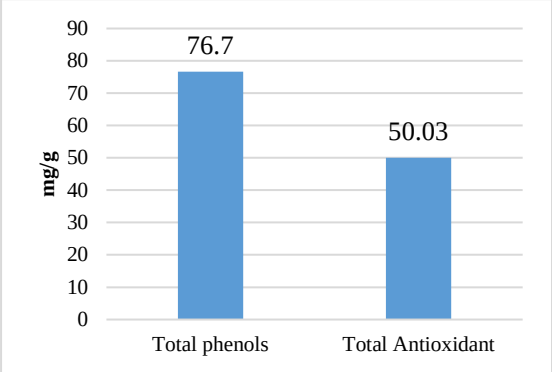

Figure (3): Total phenolic content (TPC) and Total antioxidant capacity of ethanolic extract

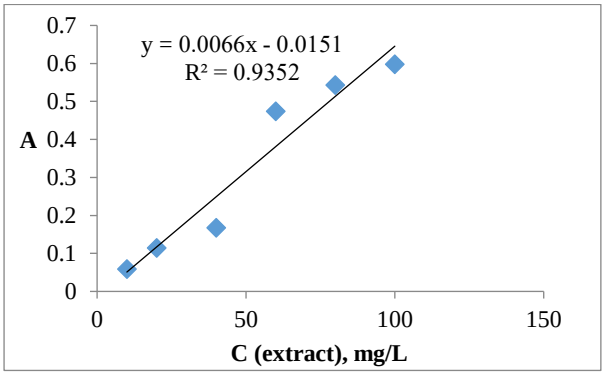

Figure (4): Variation of phenolic content with ethanolic extract concentration of *Rosmarinus Officinalis* L

3.5 Total Antioxidants

To better capture the collective impact of phenolics, flavonoids, and other reducing compounds in plant extracts, the total antioxidant capacity is often expressed in terms of ascorbic acid equivalents (AAE) (Prior & Cao, 1999). The phosphomolybdenum method is commonly used to determine the total antioxidant capacity, as it measures the reduction of Mo (VI) to Mo (V) through antioxidant activity, resulting in the formation of a green phosphate-Mo (V) complex with a peak absorption at 695 nm (Prieto & Aguilar, 1999). The total antioxidant capacity of each extract was determined based on a calibration curve (y=0.0245x–0.004, R^2=0.9808) of ascorbic acid in the concentration range of 2.0-20.0 mg/L (Figure 5), and expressed in AAE per gram dry extract weight. The ethanolic extract exhibited the highest antioxidant activity, with a total antioxidant capacity of 50.03 mg AAE/g extract (Figure 3). Additionally, there was a significant positive linear correlation between the concentration of extracts and their antioxidant activity, as shown in Figure 6.

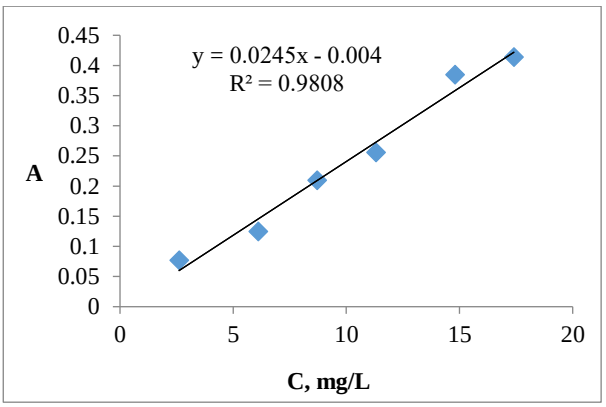

Figure (5): Calibration curve for ascorbic acid for phosphate molybdate test

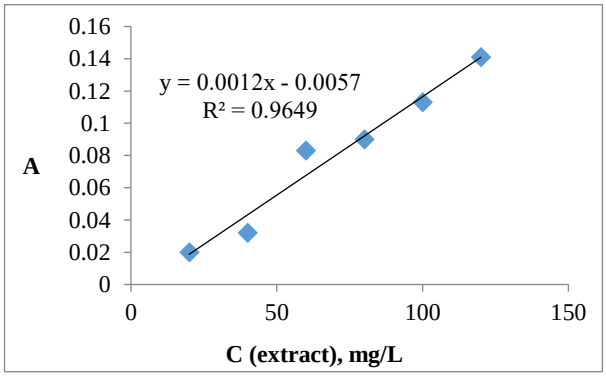

Figure (6): Antioxidant activity contents of various ethanolic extract concentrations of *Rosmarinus Officinalis* L

(Karami *et al.*, 2011) reported a total antioxidant capacity of 28.5 mg AAE/g for the methanolic extract of *Rosmarinus Officinalis* L. This value is lower than the value obtained in our study, which could be due to differences in the extraction method, solvent, and plant material used. (López *et al.*, 2006) evaluated the antioxidant activity of six essential oils, including *Rosmarinus Officinalis* L, using the DPPH assay. They reported an I_{C50} value of 46.2 ± 0.5 µg/mL for the essential oil of *Rosmarinus Officinalis* L. Although this value cannot be directly compared to the total antioxidant capacity value obtained in our study, it suggests that *Rosmarinus Officinalis* L possesses strong antioxidant activity. (Hosseini *et al.*, 2010) measured the total antioxidant capacity of *Rosmarinus Officinalis* L extract using the FRAP assay and reported a value of 41.7 ± 4.6 µmol $FeSO_4$/g. This value is comparable to the value obtained in our study and supports the notion that *Rosmarinus Officinalis* L possesses significant antioxidant activity.

3.6 Minerals Analysis

The concentrations of macroelements and heavy metals in the studied plant are presented in Table 2, with values expressed as mg/kg of sample. Macro elements,

including Na, K, Ca, Mg, and P, were found to have the highest concentrations, while Fe had the highest concentration among the heavy metals, with Cu having the smallest concentration. Specifically, the levels of Ca, Mg, Na, K, P, Fe, Cu, and Zn were 4603, 1990, 5445, 12155, 1760, 56.19, 14.38, and 18.28 mg/kg, respectively. These findings may be explained by factors such as soil composition, climate, and plant genetics (Houba *et al.*, 1990).

Table (3): Major and minor metal levels in *Rosmarinus Officinalis* L.

Metal	Content (mg/kg)
Ca	4603
Mg	1990
Na	5445
K	12155
P	1760
Fe	56.19
Cu	14.38
Zn	18.28

Previous studies have reported varying concentrations of these macro elements and heavy metals in different plant species. For example, a study by (Bhat & Mallya, 2016) reported concentrations of Na, K, Ca, Mg, P, Fe, Cu, and Zn in the leaves of different medicinal plants, including *Rosmarinus Officinalis* L. The study found that the concentrations of these elements varied widely across the different plant species, with *Rosmarinus Officinalis* L having relatively high concentrations of K and Ca. Similarly, a study by (Sousa *et al*., 2014) evaluated the levels of macroelements and heavy metals in different medicinal plants, including *Rosmarinus Officinalis* L. The study found that the concentrations of these elements varied depending on the plant species and the location where they were grown. (Ghasemi et.al., 2012) measured the concentrations of heavy metals, including Cd, Pb, Ni, and Cr, in the leaves of *Rosmarinus Officinalis* L collected from different regions of Iran. The authors reported that the concentrations of these heavy metals were below the maximum permissible limits set by the World Health Organization.

Overall, the concentrations of macroelements and heavy metals found in *Rosmarinus Officinalis* L in this study are consistent with previous reports in the literature and may be influenced by various environmental factors. These findings highlight the importance of understanding the elemental composition of medicinal plants, as they can have significant implications for their potential therapeutic and toxicological effects.

4 Conclusion

Based on the results of this study, it can be concluded that *Rosmarinus Officinalis* L. is a rich source of phytochemical constituents, total phenols, total antioxidants, and mineral contents. The plant was found to contain a variety of bioactive compounds, including flavonoids, tannins, and alkaloids, which have been shown to possess numerous health-promoting properties. The total phenolic content of the plant was found to be high, indicating that *Rosmarinus Officinalis* L. has strong antioxidant activity. This is supported by the total antioxidant capacity value obtained in the study, which was found to be 50.03 mg/g. The high mineral content of the plant, including macro elements such as Na, K, Ca, Mg, and P, suggests that *Rosmarinus Officinalis* L. could be a valuable dietary source of essential nutrients. The findings of this study provide further support for the potential health benefits of *Rosmarinus Officinalis* L. as a natural source of bioactive compounds. Further research is needed to fully elucidate the therapeutic properties of the plant and to explore its potential use in the prevention and treatment of various diseases.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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A Comparison of the Effectiveness of Artificial Neural Network Models for Time Series Data Prediction

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A B S T R A C T

Stock market prediction has become an important research area. During the last few years, various Artificial Neural Networks (ANNs) models have been proposed for stock market prediction problems. This study aims to compare the prediction performance of three artificial neural network techniques, i.e., Back Propagation Neural Network (BPNN), Radial Basis Function Neural Network (RBFNN), and Long Short-Term Memory (LSTM) in predicting the close prices of the next day. The data used in this study includes the daily close prices of two companies, Microsoft (MSFT) and Apple Inc. (AAPL), belonging to the NASDAK-100 stock exchange. The models were employed using Python software, a single hidden layer prediction model was constructed, and the effect of prediction accuracy on the number of neurons was identified. The performance of the models is measured in terms of their Root Mean Squared Error (RMSE), Mean Absolute Error (MAE), Mean Absolute Percentage Error (MAPE), and R-squared (R^2) score values. The experimental results indicated that the LSTM forecasting model outperformed alternative models with a high degree of accuracy and was found to be very efficient in learning time series data.

1 Introduction

Stock market prediction is an attractive topic for investors and researchers and has become an essential component in the financial field. Stock market prediction aims at building models based on past prices to simulate the future price, which is important for financial organizations It plays an increasingly important role in that it has a great impact on leading a proper decision on financial institutions, thus making a better profit. However, predicting the stock price itself is a challenging task due to its complex characteristics; stock markets are dynamic, nonlinear, complicated, nonparametric, chaotic, and exhibit wide variation (Binkowski et al., 2018, Shah et al., 2019). Accordingly, various solution techniques have been proposed for obtaining accurate prediction results over the years. Early techniques involved statistical methods such as Autoregressive Moving Average (ARMA) model (Rubi et al., 2022, Moradi et al., 2021) and Logistic Regression (LR) (Chen et al., 2020) (Mansouri et al., 2016). Such techniques treat the stock price movement as a function of a time series and are solved as a regression problem. However, the stock market has high volatility in nature. These statistical methods may suffer from difficulty in revealing the internal laws of the stock market. With recent developments in Computer Science, more developing techniques have been proposed to analyze nonlinear relationships in financial time series using Artificial Intelligence (AI) techniques.

Most previous studies in this area showed that AI techniques such as Artificial Neural Networks (ANN) (Gao et al., 2020), Fuzzy Logic (FL)(Hašková et al., 2023), Genetic Algorithm (GA) (Jafari et al., 2019), Support Vector Machine (SVM)(Kurani et al., 2023) are found to be more efficient than statistical methods. AI techniques have the capability of detecting the structures and nonlinear patterns of data (Mokhtari et al., 2021,

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Chen et al., 2018). In the most existing prediction methods, Neural Networks (NNs) models have been used in numerous studies for stock price prediction, and have revealed the superiority in solving nonlinear problems(Vijh et al., 2020, Nikou et al., 2019) (Siami-Namini et al., 2018, Moghar and Hamiche, 2020, Vijh et al., 2020). This advantage comes from the capability of NNs to model nonlinear techniques without previous information about the processing techniques, which can be very powerful for predicting the stock market. ANN has the benefit of storing experiential knowledge and making it accessible for use. Additionally, it has the advantages of automatic learning of features, high generalization and identification of unseen data. Thus, ANN has attracted the attention of many researchers. The first contribution of this study is the development of three artificial neural networks models, namely, Back Propagation Neural Network (BPNN), Radial Basis Function Neural Network (RBFNN), and Long Short-Term Memory (LSTM) for stock market prediction problems. The second contribution of this work is the comparison of forecasting performance of the proposed models, our aim is to verify which method is effective in predicting next day closing prices. This study focused on the daily stock close prices of two companies i.e., Microsoft (MSFT) and Apple Inc., which are from January 26, 2017, to December 26, 2020. The data of our experiment was collected from the Yahoo Finance website. Our dataset is divided into training sets which is used to train the model and update the model parameters, and test sets which would be used for testing so that we can use the data to optimize the model for data prediction. Similar input datasets were used to enable comparison between the proposed models. The performances were evaluated based on four metrics: Root Mean Squared Error (RMSE), Mean Absolute Error (MAE), Mean Absolute Percentage Error (MAPE), and R squared (R2) score values. The rest of this paper is structured as follows. A literature review of the related studies is presented in Section 2. The descriptions of the artificial neural network techniques are given in Section 3. Martials and methods of our research are described in Section 4, we introduce our dataset and describe the evaluation indicators of our experiment, and we explain our experimental procedures. The experiential results and discussion are detailed in Section 5, a comparison of the effectiveness of the proposed methods based on obtained results explained in this section. Finally, the paper is concluded in Section 6.

2 Related Work

The effectiveness of neural networks for stock market prediction problems has been examined for many years. Prediction of stock price movements was explored in (Ramesh et al., 2019), the authors presented a detailed analysis to show the efficiency of Back Propagation Neural Network (BPNN) model in predicting stock returns. They explained the importance of the choice of activation function, learning rate, and the number of neurons in the hidden layer to increase the performance of the BPNN. The results of their work were compared with a Multiple Linear Regression (MLR) model, and they found that the BPNN based model gave better results in predicting stock returns with good accuracy. The authors in (Song et al., 2018) evaluated the performance of neural network models in stock market index perdition using adjusted close prices of three different stocks, namely Bank of China, Vanke A, and Kweichou Moutai. They reported a comparison between BPNN, Radial Basis Function Neural Network (RBFNN), General Regression Neural Network (GRNN), Support Vector Regression (SVR), and Least Squares Support Vector Regression (LS-SVR). The performance of the proposed models is evaluated using statistical indicators such as Adopting Mean Square Error (AMSE) and Mean Absolute Percentage Error (MAPE). They observed that the BPNN model outperforms the other four models. The performance of ANN was also investigated in (Mansor et al., 2020). Their work aims to compare the predictive performance of five neural network architectures, namely: Multiple Linear Regression (MLR), Elman Neural Network (ENN) Elman, Jordan Neural Network (JNN), Radial Basis Function (RBF), and Multilayer Perceptron (MLP), in predicting six traded stocks of the Brazilian stock exchange. They use the test data to tune the number of input variables and suitable hidden layers. The models were trained to predict the closing price of the next day from the previous values. The performance of all fitted models was assessed by the Root Mean Square Error (RMSE). In their results, they found that the ENN, JNN, MLP, and MLR networks presented quite similar RMSE for all times series analyzed in their research and the MLR may be tuned to provide results quite similar to more complicated models such as the MLP, ENN, and JNN neural networks, since it provides the best result .The performance of the neural network was also investigated in (Moghar and Hamiche, 2020) using LSTM for forecasting two stocks on the New York Stock Exchange (NYSE), i.e., Google (GOOG) and Nike (NKE) stock prices extracted from the Yahoo Finance website. The reported results indicated that the proposed Long Short-Term Memory (LSTM) model could improve forecasting accuracy and is capable of tracing the evolution of stock prices for both stocks. A comparison study was reported in (Tiwari et al., 2020). The authors compared ANN and Fuzzy Logic (FL) in a problem of stock market prediction using the daily historical prices listed on the Bombay Stock Exchange (BSE). A Back Propagation (BP) algorithm was used to train the neural network. The experimental results of their work revealed that the neural network gave better prediction results in terms of the minimum value of mean square error. The authors in (Nabipour et al., 2020) conducted a comparison study between Decision Tree

(DT), Random Forest (RF), Adaptive Boosting (Adaboost), Extreme Gradient Boosting (XGBoost), Support Vector Classifier (SVC), Nave Bayes (NB), K-Nearest Neighbors (KNN), Logistic Regression (LR), and ANN and two deep learning methods, Recurrent Neural Network (RNN) and LSTM. Ten years of historical data from four stock markets, namely Petroleum, Diversified Financials, Basic Metals, and Non-Metallic Minerals, were used. Ten technical indicators from the selected historical data were chosen as input values. They conclude that RNN and LSTM are superior models in both approaches compared with other models, and a significant improvement in the performance of models is observed when they use binary data as input values. In (Hiransha et al., 2018), four types of ANN architectures have been utilized, namely Multilayer Perceptron(MLP), Recurrent Neural Network(RNN), Long Short-Term Memory (LSTM), and Convolutional Neural Network (CNN), for predicting the stock prices of two different stock markets, the National Stock Exchange (NSE) of India and the New York Stock Exchange (NYSE). The authors compared the obtained results with the Autoregressive Integrated Moving Average (ARIMA) model. The results show that the neural network models outperform the ARIMA model. Another study is discussed in (Botunac et al., 2019). The performance of three different neural network models was compared on financial data using the Feed Forward Neural Network (FFNN), Recurrent Neural Network (RNN), and Long Short-Term Memory (LSTM). In their study, the data were preprocessed with a simple moving average and filtered with a discrete wavelet transformation to achieve better results. Among the tested neural network models, better results were obtained using LSTM than other neural network models. A comparison between Autoregressive Integrated Moving Average (ARIMA), Back Propagation Neural Network (BPNN), and Genetic Algorithm (GA) was conducted by (Alfred et al., 2015) to predict the short-term time series network traffic activity datasets, which were obtained from the ICT Universitas Mulawarman. The performances of these models are compared based on mean squared error. Based on the results obtained, BPNN is found to be very efficient in capturing the structural relationships in time series data. Another study reported by (Nikou et al., 2019), the study compared the performance of Multilayer Perceptron (MLP), Long Short-Term Memory (LSTM), Random Forest(RF), and Support Vector Regression (SVR) with daily closing values of iShares MSCI United Kingdom exchange rates. They report that the LSTM model is a better predictive model in the prediction of the close price of iShares MSCI United Kingdom.

3 Background

In this section, we review the basic concepts of the underlying technologies used in this study.

3.1 Back Propagation Neural Network

The Back Propagation Neural Network (BPNN) is a machine learning algorithm, that aims to minimize the mean square error gradually between the actual and target outputs of the network .The common structure of BPNN model is illustrated in Fig. 1.

As viewed in architecture, there are three layers: an input layer, a hidden layer, and an output layer. The input layer is connected to the hidden layer by interconnection weights, and the hidden layer is connected to the output layer by interconnection weights**.** Each layer has a number of nodes and each node represents a neuron.

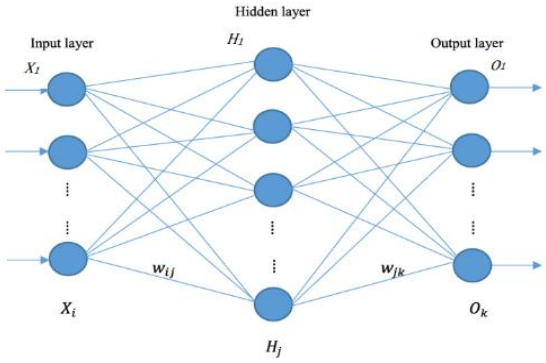

Figure (1). Back Propagation Neural Network Architecture

BPNN requires a suitable choice of network architecture, which is the number of neurons required to form the network. It is important to identify the total number of neurons in the hidden layer. Selection of the neurons is done by the trial and error method. A small number of neurons may result in incorrect estimation whereas more neurons result in overestimation. In addition, the formation of the network requires a proper choice of network layers. Increasing the number of layers increases the computational complexity of the neural network, which can increase the time taken for convergence. Each hidden neuron in BPNN performs a weight summation operation. The outputs of all hidden layer nodes are calculated as follows:

$$h_j = f\left(\sum_{i=1}^{n} w_{ij} x_i\right) \quad (1)$$

where i is the input node (i=1,2,…,n), j is the hidden layer node (j=0,1,2,…,m), w_{ij} is connection weight from input node i to hidden node j, h_j is the output of the jth node in the hidden layer, and f is the activation function of nodes. In our an model, we use the sigmoid function as activation function, which is defined as

$$f(x) = \frac{1}{1+exp(-x)} \quad (2)$$

A suitable selection of the activation function is a critical step that introduces nonlinearity into the network for the hidden layer, because it provides the ability of the network to capture the nonlinear relationship between input and output. Several BPNN training algorithms have been proposed for adjusting the connection weights, such as Gauss-Newton's algorithm (Nandy et al., 2012) and the Levenberg–Marquardt algorithm (Mustafidah et al., 2019). All algorithms use the gradient of the performance function to determine the adjustment of the weights to minimize the performance. That is the Widrow-Hoff learning rule, in which the network weights are moved along the negative of the gradient of the performance function, which is the steepest descent direction. The training process of the network occurs in the following steps. In the first step, the training samples were entered into the input layers. Then, the presented data propagates through the hidden layers to the output layers. In the second step, the errors are calculated by taking the difference between the actual and desired outputs. The network weights will be continuously adjusted by propagated back network error until the desired output error is obtained, which represents the minimum output error. This means that the smaller the output error, the better fit the model. Python software provides effective machine learning libraries for building and optimizing neural network models which, has been considered in this study. The formula for the BP algorithm is described by the following equations:

$$W(n) = W(n-1) - \Delta W(n), \quad (3)$$

where,

$$\Delta W(n) = \gamma \frac{\partial E}{\partial W}(n-1) + \theta \Delta W(n-1), \quad (4)$$

where γ is the learning rate, E is the gradient of error function, and $\theta \Delta W(n-1)$ the quantity of incremental weight.

Back propagation networks have the ability to learn complicated multidimensional mappings. It can be used to simulate nonlinear mapping models, solve some real-world problems, such as classification, and prediction; it is a well-known, powerful tool in problem solving for various stock price predictions.

3.2 Radial Basis Function Neural Network

A Radial Basis Function Neural Network (RBFNN) is a type of feed forward neural network with the use of radial basis functions as activation functions. The basic structure of RBFNN is shown in Fig. 2.

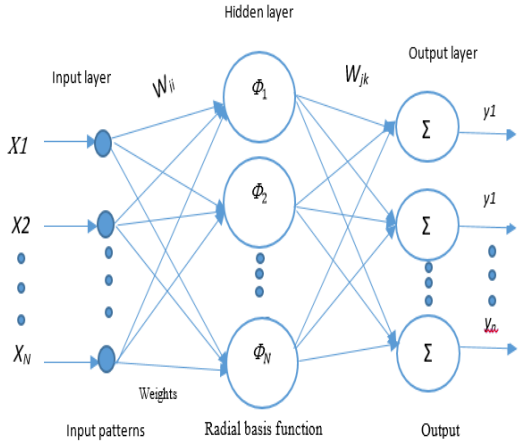

Figure (2). The basic structure of an RBF neural network

Typically, the network has three layers: an input layer, a hidden layer with activation nodes based on the Radial Basis Function (RBF), and a final output layer. Similar to the typical ANN structure, each layer consists of a set of nodes. The input layer receives the input vector and transforms its value to the hidden layer. Each node in the hidden layer has its own RBF centered at a point and is connected to the input layer. The output layer produces the outcome of the prediction model. The output of the RBFNN is defined as a weighted average of the incoming signals from the hidden layer. In practice, the structure of RBFNN may require more neurons in a hidden layer than Feed Forward Neural Network (FFNN). The number of hidden neurons in the hidden layer is critical for determining the RBF model capability.

The nonlinearity of the RBFNN model is presented by mapping the input data to hidden layers using the basis function, whereas a linear mapping appears from the hidden layers to the output layer. The activation function of the RBFNN can take different types of radial basis functions. A Gaussian activation function has been commonly used as an activation function in the hidden neurons of the RBF model. However, it is difficult for the Gaussian activation function to approximate constant values, and the models may suffer from an approximation of these values. A sigmoid function as the basis function of the network may replace the Gaussian functions, so more accurate results can be found by an RBF network(Wu and Wilamowski, 2013) . In our model, a sigmoid function was used to deal with this problem. When the sigmoid function is used, the common form of the kth node of the output layer that has the weight W_k and the neuron *i* of the hidden layer is as follows:

$$y_k = \sum_{i=1}^{n} w_{ki} exp\left(\frac{1}{1+exp(-x)}\right), k = 1,2,3,..,q \quad (5)$$

RBFNN generally trains faster than BPNN due to the use of the radial basis functions and can effectively fit any

nonlinear function, and it is not easy to fall into the local optimal solution; RBFNN can improve the accuracy and decrease the training time complicity.
RBF networks have many uses, such as time series prediction (Sohrabi et al., 2023), system control (Mehrsai et al., 2013), and classification(Jiang and Li, 2019). Many applications have shown that RBFNN can be a useful method for the stock market prediction. This is due to its ability to perform universal approximation and regularization, it can approximate any continuous function with high precision (Liao et al., 2003).

3.3 Long Short-Term Memory

Long Short-Term Memory (LSTM) neural network is an improved type of RNN with better performance in long-term dependency in many applications (Qu and Zhao, 2019). RNN is a form of neural network that has the ability to process sequential inputs recurrently due to the internal time loops at each hidden layer unit in which the output of the unit at a specific time step is taken as the input for the next time step. However, the RNN has a limit in real practice, in that it is just able to record the information in a few past steps. It appears to have vanishing gradient problems when dealing with long time series data. The LSTM units have been proposed to address the vanishing gradient problem where the hidden layer is replaced by recurrent gates called forget gates. These gates allow modelling of long-term dependences in sequence data and prevent the vanishing gradient problem (Gers et al., 2000, Graves and Graves, 2012, Nugaliyadde et al., 2019). LSTM networks are adapted for identifying long-term dependencies and using them for future prediction, which was impossible in a standard RNN architecture where the network could just learn a limited number of short-term time series. The key element of the LSTM is the appending of cell state or a memory cell, which **is** comprised of three basic gates: an input gate, a forget gate, and an output gate. The forget gate decides which information from the previous cell is completely to be kept or ignored. The input gate chooses which new values need to be updated in the cell state, and the output gate lets the cell state have an impact or not on the latest present time step**.** LSLM also has a number of hidden units.

The sigmoid neural net layer and weights are used to assign importance to information. The stochastic gradient descent based algorithm is used to enforce constant error propagation (neither exploding nor vanishing) through its internal units. Fig.3 shows the architecture of the LSTM**.**

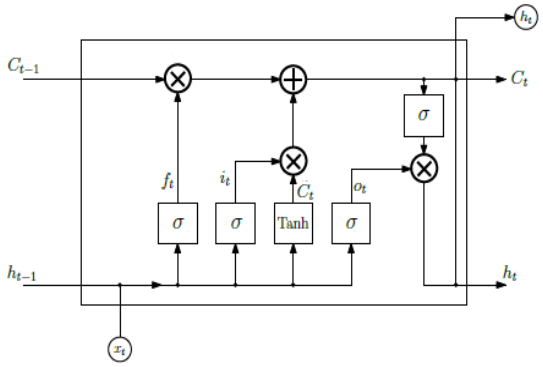

Figure (3). Basic architecture of a LSTM

The nature of the three basic gates of the LSTM can be described in the following steps: In the first step, the forget gate has to decide which information to discard from cell state. The formula of the forget gate is characterized by the following equations:

$$f_t = \sigma\left(W_f . [h_{t-1}, x_t] + b_f\right) \quad (6)$$

$$R_t = \frac{1}{1+e^{-t}} \quad (7)$$

where h_{t-1}, x_t , $R_t$and f_t represent the output at the previous time (*t-1*), input at the present timestamp (*t*), a Sigmoid function and a forget gate, respectively, W_f and $b_f$are the weight matrices and bias vectors, respectively, that need to be learned during the training process.
In the second step, the input gate layer decides which new information should be saved in the cell state. The input gate has the following mathematical representation:

$$i_t = \sigma(W_i . [h_{t-1}, x_t] + b_i \quad (8)$$

$$\tilde{C}_t = tanh(W_c . [h_{t-1}, x_t] + b_c) \quad (9)$$

Updating the information stored in the cell state has been done by the following equation:

$$C_t = f_t * C_{t-1} + i_t * \tilde{C}_t \,, \quad (10)$$

where C_t, C_{t-1} and $\tilde{C}_t$ represents the current cell state value , the last timestep cell state value, and an update for the current cell state value at timestamp *t* respectively.

In the third step, the output gate layer determines the output information. The characteristics of the output gate layer are expressed in the following equations:

$$O_t = \sigma(W_o . [h_{t-1}, x_t] + b_o) \,, \quad (11)$$

$$h_t = O_t . tanh(C_t) \,, \quad (12)$$

where O_t is the output gate that indicates the candidate for cell state at timestamp *t* and h_t is the LSTM block's output information at time *t*.

Building an LSLM model includes the following steps:
Step1: The collected data is preprocessed to eliminate any undesirable or incomplete data.
Step2: Divide the data into training and testing data. The training data is used to build the model and the test data to evaluate the model's performance.
Step3: Selection of a suitable architecture for the LSLM Model. This step requires an appropriate choice of the number of the nodes and layers.
Step4: Train the model by repeating the training cycle until the desired result is obtained.
Step5: Making predictions and assessing the model's performance.

4 Materials and Methods

4.1 Dataset

The historical data of the daily stock close prices for two different well-known NASDAQ-100 companies has been chosen to represent our dataset, namely Microsoft Corporation (MSFT) and Apple Inc. (AAPLE). Our dataset is taken from the Yahoo Finance website and includes four years of data, which is from January 26, 2017 to December 26, 2020. Fig.4 shows two different closing stock prices.

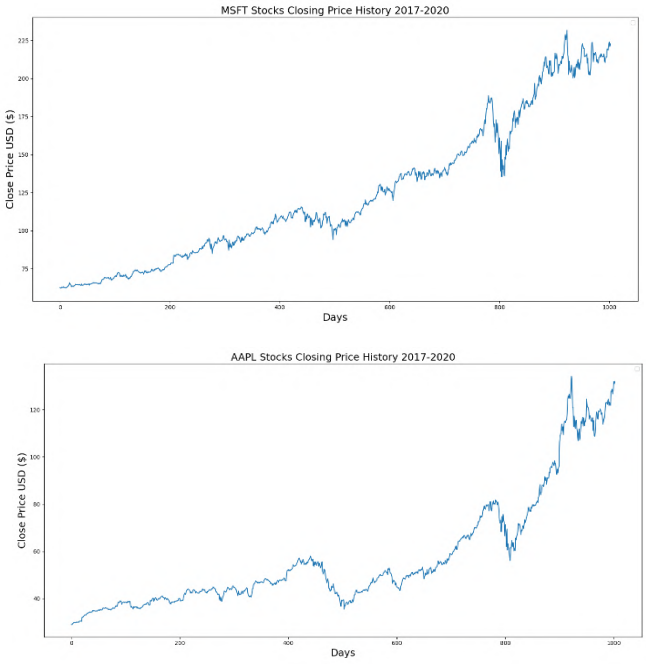

Figure (4). Closing stock prices for MSFT and AAPLE

Before using the dataset for the training process, it is important to take a preprocessing step. The preprocessing step used in this study is data normalization using the equation formula:

$$Z'_i = \frac{Z_i - min(Z_i)}{max(Z_i) - min(Z_i)} \quad , \qquad (13)$$

where $\boldsymbol{Z'_i}$ is normalized values, $min(Z_i)$ is the minimum value of input $\boldsymbol{Z_i}$, and $\boldsymbol{max}(\boldsymbol{Z_i})$is the maximum value of input $\boldsymbol{Z_i}$.
The normalization process aims to make data statistically comparable. This can also help the learning process be more stable. Data normalization helps to smooth the convergence, which prevents dramatic changes in the gradient. After processing, we anti-normalize the output with the following equation:

$$\widehat{y_t} = y'_t(y_{max} + y_{min}) + y_{min} \, , \qquad (14)$$

where y'_t represents the predicted data after anti-normalization and y_{max} and y_{min} the minimum and maximum data, respectively, of output y'_t.

Our dataset was divided into 90:10 ratios for training and testing purposes, respectively. For all models, the training dataset is used to train the neural network models and update model parameters. The test dataset was used to optimize the models for data prediction, in other words, the test dataset was used for simulating the trained network and checking the accuracy of the trained network. The accuracy of the model on the test dataset gives you a very rough estimate of how accurate the model will be when presented with new, previously unseen data.

4.2 Model Design

Our aim looks at the comparison of the three NNs models BPNN, RBFNN, and LSTM in a problem of stock market prediction. The basic principles of them have been detailed in the previous section. The process of training algorithms is described later in the paper. After training the proposed models and evaluating their accuracy, we compared the output data given by the network with the testing dataset. The results of these comparisons are given in detail in the later part of this paper. Fig.5 shows the block diagram of the methodology.

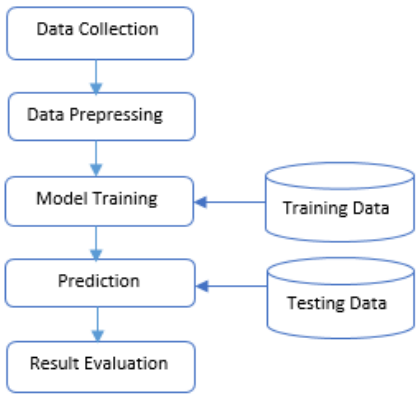

Figure (5). Methodology block diagram

4.3 Performance Evaluation Criteria

We select the following statistical metrics to evaluate the predictive performance of proposed models: Mean Absolute Error (MAE), Root Mean Square Error (RMSE), Mean Absolute Percentage Error (MAPE), and coefficient of determination (R2). MAE is the measure of the deviation between the actual and predicted values. MAPE is commonly presented the accuracy of the model as a percentage in which equation (16) is multiplied by 100. The statistical matrix RMSE measures the mean square error of the actual and predicted values; its value is always positive and zero in the ideal case. The lower the MAPE, MAE and RMSE values, the closer the predicted time series values are to actual values, indicating that the model is accurate. R2 also gives the accuracy of the model as percentage. The smaller values present the better predictor model; it gives you knowledge of how well the model predicts the new dataset. Its values are between zero and one, and the largest value is the better value. The equations for these criteria are as follows:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_t - y_t')^2}, \quad (15)$$

$$MAPE = \frac{1}{n}\sum_{i=1}^{n}\frac{|y_t - y_t'|}{y_t} \times 100, \quad (16)$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\frac{|y_t - y_t'|}{y_t} \quad (17)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_t - y_t')^2}{\sum_{i=1}^{n}(y_t')^2}, \quad (18)$$

Where

y_t is the actual value at the time *t*
y_t' is the forecast value at the time *t*
n is the total number of tested datasets

4.4 Experimental implementation

All experiments were conducted in Python with PyCharm Professional Edition 2020.2.1 on an Intel Core i5-5200U CPU machine. The implementation includes construction, training, testing, and evaluation of neural network models. All neural network models were developed using the open source deep learning tool Tensorflow (Abadi et al., 2016) with Keras (Ketkar, 2017) version 2.0.8 as the front-end interface. The Adam optimizer was applied to all of the neural network models for weight modification and Mean Square Error (MSE) was used as the cost function. The formula for MSE is given as follows:

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(y_t - y_t')^2, \quad (19)$$

where $\mathbf{y_t}$ and $\mathbf{y_t'}$ are actual and predicted values at time ***t***, respectively, and ***n*** is the data size of the trained set.

To ensure keeping the same conditions when training different models, a single hidden layer was used in all prediction neural network models and one fully connected layer has been used as the output layer, which gives the predicted next day value. Furthermore, the batch size is considered as 32, and epochs are kept constant at 200 for all prediction models. To evaluate the performance of the models, we used the prediction accuracy indicators. The accuracy indicators tell us how accurate the forecast model is at predicting future trend movements**.** To indicate the performance of the ANN models, the optimal number of hidden layer neurons should be decided in ANNs. We start our implementation with the first method of PBNN. A number of hidden layer neurons have been tried, which are illustrated in Table 1 for two datasets. A sigmoid transfer function was used in the hidden layers, and a rectified linear unit activation function was used in the output layer. The optimal number of hidden layers of neurons is based on the smallest MSE value generated, which is shown in bold in Table 1. It is noticed that as the number of hidden neurons increases, the mean square error increases gradually. The second method is RBFNN; we start the experiment by determining the optimal number of hidden neurons in the network architecture. A number of hidden layer neurons were modified, and Table 2 shows the results of this experiment for two data series. The value of the optimum hidden neuron that was chosen in this study is given in bold in the table with the smallest test mean square error value. Furthermore, the output layer of transform functions was chosen to be the sigmoid transfer function in the RBF model**.**

Table (1). The result of the determination of the number of neurons in the hidden layer of PBNN

No. of neurons	MSE	
	AAPLE	MSFT
100	**0.000105**	**0.000108**
200	0.000340	0.000120
300	0.001482	0.000407

Table (2). The result of selecting the optimal number of neurons for the RBFNN's hidden layer

No. of neurons	MSE	
	AAPLE	MSFT
100	**0.000184**	**0.000200**
200	0.000208	0.000209
300	0.000220	0.000261

The third model is the LSTM model. Similar to previous methods, the optimal number of neurons should be specified. In this model, a sigmoid activation function was used in the LSTM cells. The optimum number of neurons has been obtained after trying different values and the results of the experiment are presented in Table 3. The optimal number is given in bold in the table**.** Furthermore, we used the dropout method on all layers of the network to prevent overfitting during network training and improve generalizability. The dropout units technique in a neural network depends on the value of the probability ***p*** of retaining a hidden unit in a neural network. To obtain the optimal value of *p*, which is a hyperparameter in the dropout, we tried different values of ***p*** ranging from 0.2 to 0.6 after considering the optimal number of the neurons as 300 in the LSTM model and 100 neurons in BPNN and RBFNN models, then we chose the best value of ***p*** that gives the best accuracy with a small amount of error. The results are given in Table 4, which indicates that the best accuracy is obtained with ***p*** = 0.2 in the BPNN and LSTM models, whereas the dropout with the probability of 0.4 in the RBFNN model has fewer errors, thus has been considered the optimum value of *p* in RBFNN.

Table (3). The result of selecting the LSTM's optimal number of neuron.

No. of hidden layer	Number of neurons	MSE	
		AAPLE	MSFT
1	100	0.000183	0.001925
1	200	0.000153	0.001326
1	**300**	**0.000140**	**0.001100**

Table (4). The result of estimating the optimal dropout rate *p*

Dropout rate *p*	MSE					
	PBNN		RBFNN		LSTM	
	AAPLE	MSFT	AAPLE	MSFT	AAPLE	MSFT
0.2	**0.000401**	**0.000350**	0.000223	0.000211	**0.000135**	**0.000841**
0.3	0.000941	0.000474	0.000151	0.000179	0.000322	0.001344
0.4	0.001048	0.002277	**0.000130**	**0.000162**	0.000281	0.001915
0.5	0.003536	0.002015	0.000148	0.000183	0.000422	0.002907
0.6	0.005195	0.005153	0.000161	0.000150	0.000683	0.003288

5 Results and Discussion

Table 5 shows the summarized experimental results of three neural network models on the two different datasets; the best performing model is displayed in boldface. By comparing the statistical errors for all models, the LSTM model achieves the lowest RMSE and MAPE, and the highest R^2 on the prediction of both datasets. However, in terms of MAE, RBFNN reported the smallest errors value of 0.009586 on the prediction on AAPLE dataset, while on the prediction of MSFT dataset, LSTM has a better performance. LSTM model performed well because it does not depend on any previous information for prediction, which enables the model to understand the dynamic changes and patterns occurring in the current window. This can be beneficial for non-stationary time series such as stock market. RBFNN model also performed well and has very close results to LSTM model. However, in the case of BPNN, the model has the worst performance across two stacks. This is due to its simple architecture, which could limit its capability to make predictions for non-stationary time series. We could also find from Table 5 that the AAPLE models show better results than the MSFT models. The AAPLE models have the smallest values of RMSE, MAPE, and MAE and the determination R^2 has a much closer fitted result on the AAPLE dataset. Fig. 6, Fig. 7 and Fig.8 display the actual test data versus predicted test data graphs of three models on two different stock prices for one-day ahead prediction. The red lines indicate the predicted test data, and the black lines indicate the real test data for MSFT stocks. The orange lines indicate the predicted test data and the black lines indicate the real test data in the AAPLE stock prices.

It can be seen that, generally, all of these models perform relatively well, demonstrating that the past prices of stocks have predictive power and can be used to predict

future prices. The predicted output fairly overlaps the target output, indicating a good prediction. Additionally, the turning points are forecasted quite timely. When there is a trend in the actual price, the predicted value follows accordingly and closely. It is also clear that the models that were proposed using the AAPLE dataset have better fitted results than the MSFT dataset.

Table (5). Results of the three methods

Dataset		AAPLE				MSFT			
Metrics		RMSE	MAE	MAPE	R^2	RMSE	MAE	MAPE	R^2
Models	BPNN	0.024895	0.020301	4.493746	0.988906	0.036203	0.03343	4.791764	0.982899
	RBF	0.012556	**0.009586**	2.076750	0.997178	0.030193	0.015742	1.506608	0.988105
	LSTM	**0.011353**	0.010190	**1.213605**	**0.997692**	**0.013394**	**0.010510**	**1.895189**	**0.997659**

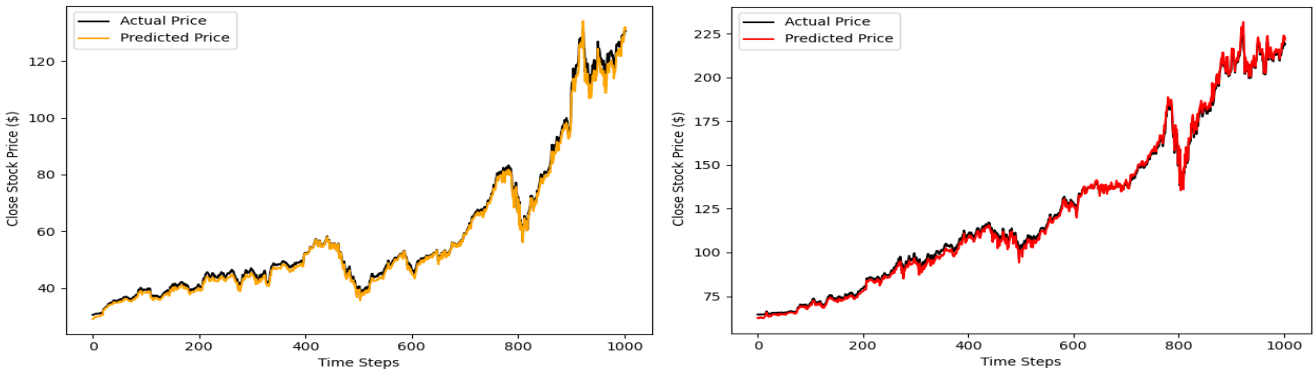

Figure (6). BPNN model predicted results.

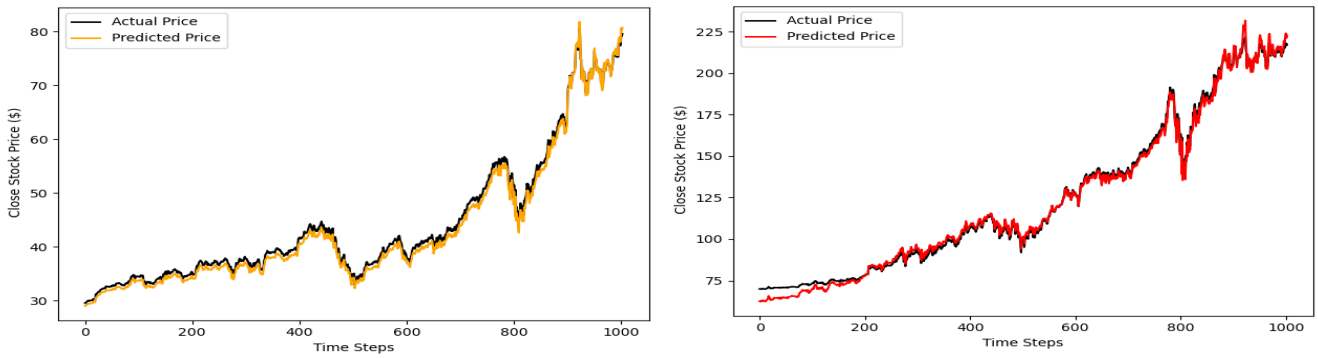

Figure (7). RBFNN model Predicted results.

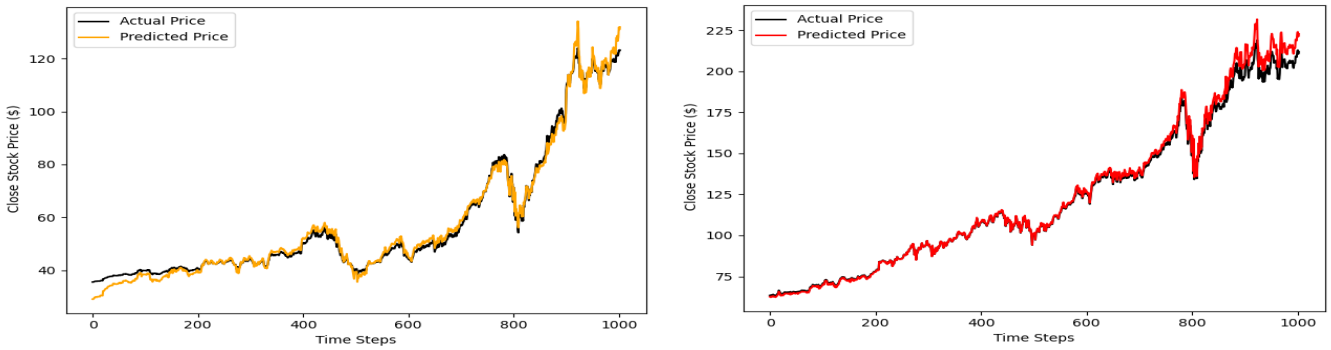

Figure (8). LSTM model predicted result.

6 Conclusions and Future Work

This study compares the performance of three neural network learning models, i.e., BPNN, RBFNN, and LSTM, by predicting movements in the one-day ahead of stock prices. The study address the following question: which neural network models provide the best predictive performance for both datasets? By implementing the proposed methods and checking the accuracy of the models using statistical errors, we conducted a comparative study between three NN models for predicting the stock market. With evidence from the forecast accuracy of two stocks' close prices. We find that all techniques perform well with acceptable accuracy, but the LSTM model beats other models in the prediction of the close prices on two datasets, and its performance was followed by that of RBFNN model. Which indicates that it may be conceivable to utilize the LSTM model as an effective approach to successfully predict the future pattern of stock prices based on the results of this study, the following recommendations can be made:

- Deep neural network models is better than the other techniques that have been utilized in this study. Researchers and investors are recommended to employ these methods for predicting the stock price.
- We recommend using hybrid models such as optimized LSTM with optimization techniques such as Particle Swarm Optimization (PSO) and combining BPNN and RBNN with other AI methods such as Fuzzy Logic (FL), Support Vector Machine (SVM), and Genetic Algorithm (GA), a lot of research work has been found that hybrid models improve stock prediction accuracy.
- The feature selection step should be taken under consideration in future work and compared with the results obtained in this study. It is suggested that the researchers use feature selection algorithms to extract the features of stock prices, such as Deep Belief Networks (DBN), the

  Discrete Wavelet Transformation (DWT) technique, Relief, maximum Relevance and Minimum Redundancy (mRMR), and LASSO. Such techniques successfully remove certain types of noise from data and improve the quality of the neural network model.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Floristic Study of Al-Orban area in Gharyan District-Libya

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A B S T R A C T

The goal of this study was to investigate the vegetation composition and structure of the Al-Orban area in Gharyan district, Libya over two continues growing seasons from the first of January 2020 to the first of Jaunary 2022. During this period, a total of 309 plant species were identified and collected in the field. These species belonged to 43 families, with 39 families of dicotyledons and 4 families of monocotyledons. The most dominant family was *Asteraceae* with 60 species, followed by *Fabaceae* with 32 species, *Poaceae* with 33 species, and *Brassicaceae* with 35 species. The most dominant genera were *Plantago* and *Euphorbia*, each represented by 6 species, followed by *Erodium* and *Chenopodiun* with 5 species each, and *Astragalus, Centaurea, Rumex, Concolvulus*, and *Stipa* each represented by 4 species. Life-form spectrum analysis indicated that Therophytes were the most predominant with 189 species, followed by Hemicryptophytes with 47 species, and geophytes with 40 species. Furthermore, chorotype spectrum analysis showed the dominance of Mediterranean species with 128 species, followed by Mediterranean/Iranian-Turanian with 66 species. These findings provide valuable insight into the plant diversity and distribution in the Al-Orban area of Gharyan district, Libya.

1 Introduction

The Mediterranean basin is recognized as one of the most regions vulnerable to climate change, with evidence of significant changes in temperature and precipitation patterns over the last century (Zommers *et al*., 2016). Dry land ecosystems, which are already subject to natural and anthropogenic pressures such as land-use change, resource demands, and population growth, are particularly impacted by these changes, leading to altered distribution patterns and increased fragmentation of landscapes (Staudinger *et al.*, 2012; Bangash *et al*., 2013). To address the urgent need for biodiversity conservation in such ecosystems, taxonomic and floristic studies have become increasingly important, providing critical data for understanding biodiversity and ecosystem functioning (Heywood, 2004).

The vegetation of mountainous regions is of particular importance due to its high biodiversity and relative density of vegetation. However, these ecosystems are at risk of degradation and loss due to anthropogenic pressures such as overgrazing and land-use change. In Libya, regions such as the Nafusa Mountains have experienced significant changes in vegetation cover over the last century, leading to the loss of natural habitats and threatened plant species (El-Ahmir *et al.*, 2020).

The present study aims to provide a comprehensive analysis of the vegetation of the Mountains and valleys of the Al 'Orban area, located in the transitional zone between the steppe and mountain regions of Gabel Nafusa. Through detailed spatial

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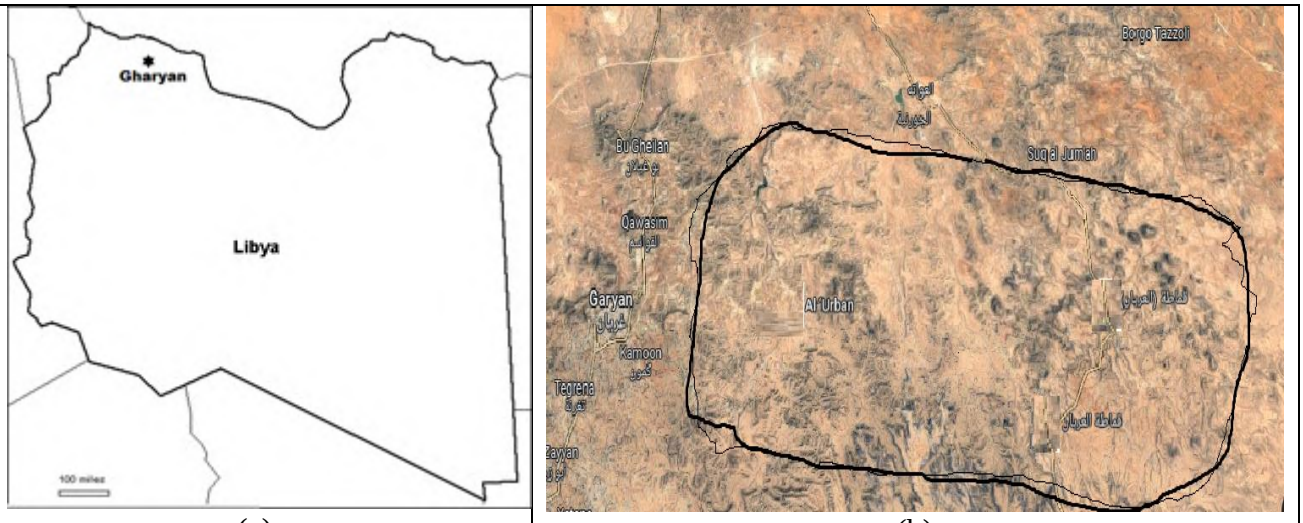
analysis, this study seeks to explore the distribution patterns and ecological features of mountainous vegetation in this region. Ultimately, this study aims to contribute to the conservation and management of natural resources in this area, and to advance our understanding of the biodiversity and ecological functioning of mountainous ecosystems in Libya.

1.1 Study Area

Al-Orban is located 130 km Northwest of the Libyan capital, Tripoli. The study area bordered to the west by Gharyan, to the south by Awlad Boussif, to the north by Tarhuna, and to the east by Bani Walid. (32º 16´65^0 latitude N and 13º 15´44 longitude E) and occupies an area approximately178 km2. It is described as a transitional zone between steppe and mountain regions (Fig 1a,1b). Climatically, this region follows the Mediterranean climate which characterized by hot and dry summers with high summer temperatures. The average annual temperature is 23°C and rainfall, on average ranges between 100-200 mm annually. December and January are the wettest months while the majority of rainfall occurs in the winter season, with the rainy season addition to the geographic and topographic variation of the region, considerable edaphic variation exists. Much of the area is covered by gravels with sandy clay subsoils at depth, also un weathered granite outcrops, zones of kaolinitic clays and areas of bleached sandy soils with compacted pan-like layers also are present (Salem & Busrewil 1980). These environmental conditions can provide considerable scope for diversity in the floristic composition, adaptive characteristics displayed by the plants, patterns of groupings of plant species and the structural features of the plant communities. Some detailed studies on the vegetation of the North part of Gharyan, were conducted by (El-Ahmir & Abuhadra, 2008), Al-Orban areas have never been investigated apart from few fragmentary and brief reports prepared by (Jafri,& El-Gadi,1976-1989). In this work, an extensive and thorough floristic survey was made, covering the area of Al-Orban Mountains Gharyan –Libya.



(a) (b)

Figure 1: (a) Showing the location of the study area, (b) Satellite image showing the studying area.

2 Materials and Methods

Due to the drought of the region this study lasted for two consecutive years from (1/1/2020 to 1/1/2022) to investigate the status of plant diversity in the study area. During this period, eighteen trips to the study area were conducted. A total number of 309 plant species were collected upon various field trips during this period, the collected plant specimens then were brought into the herbarium for further treatments. Identification of plant specimens was authenticated by the authors and confirmed by using dichotomous keys, plant description, illustrations, and photographs, provided by manuals and floras of the region, such as Flora of Libya (Jafri and El-Gadi, 1976 – 1989), Flora Palaestina (Zohary. 1966. & 1972; Feinbrun-Dutan 1976-1986)).

Flora of Egypt (Täckholm, 1974). Flora of Syria, Palestine and Sinai (Post, 1932-1933). Key to The Families of Flora of Libya (F. B. Erteb. 1994). The Grasses of Libya (Sherif, 1995). Finally, the plant specimens were deposited at the Herbarium of the botany department, faculty of sciences, University of Garyan.

3 Results and Discussion

A survey of the study area has done in the period between January 2020 and January 2022 to investigate the status of plant diversity of the study area. This survey has led to collection and identification of 309 plant species, 262 are belonging to dicotyledons and 47 species belong to monocotyledons. The collected plant species represented by 43 families where 39 families belong to Dicotyledons and 4 belong to Monocotyledons (Appendix 1).

After calculation the percentage of each family in relation to the total number recorded. The result showed a predominance of the family *Asteraceae* which itself comprise (19.4 %), with the number of 60 species, followed by the family *Poaceae* which comprise (10.7 %), with the number of 33 species, then the family *Fabaceae* which comprise (8.7 %), with the number of 27 species, followed by *Brassicaceae* which comprise (8.0 %), with the number of 25 species. The rest of the result shown in the (Fig 2).

The dominant genera recorded in this study were *Plantago* and *Euphorbia* both represented by 6 species each, followed by *Erodium* and *Chenopodiun* which represented by 5 species each, then *Astragalus*, *Centaurea*, *Rumex*, *Concolvulus* and *Stipa*, each represented by 4 species (Fig 3).

The dominance of the family *Asteraceae* is expectable because most members of this family are Therophytes which are dominating the Mediterranean region which characterizes the study area, in addition it is one of the largest families the flowering plants and with cosmopolitan distribution, so it is expected that it will occupy highest ratio.

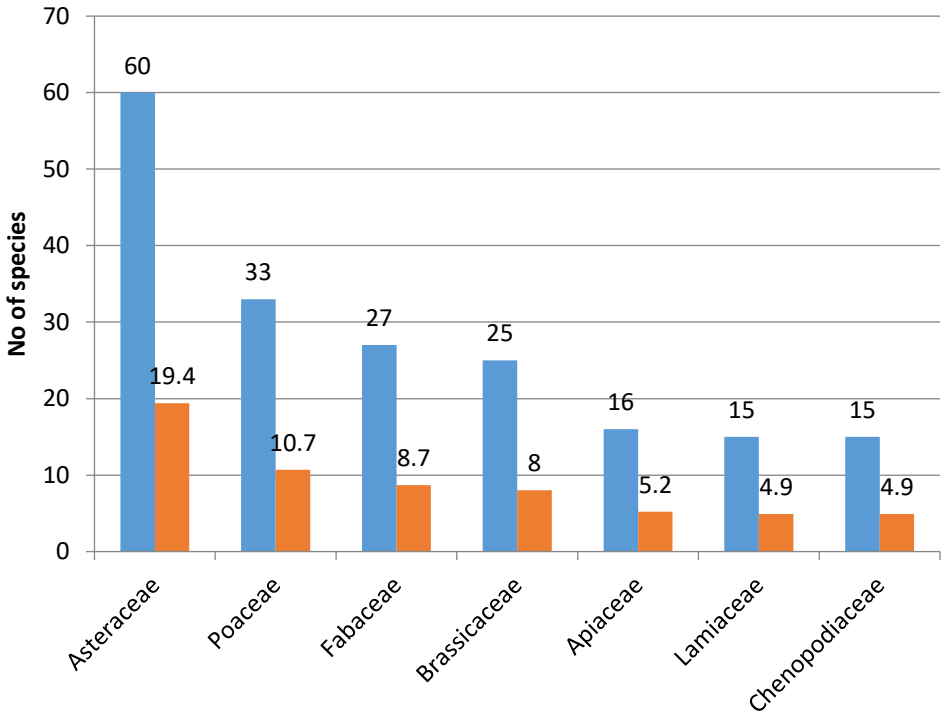


Figure 2: Dominant families with their numbers and percentages**.**

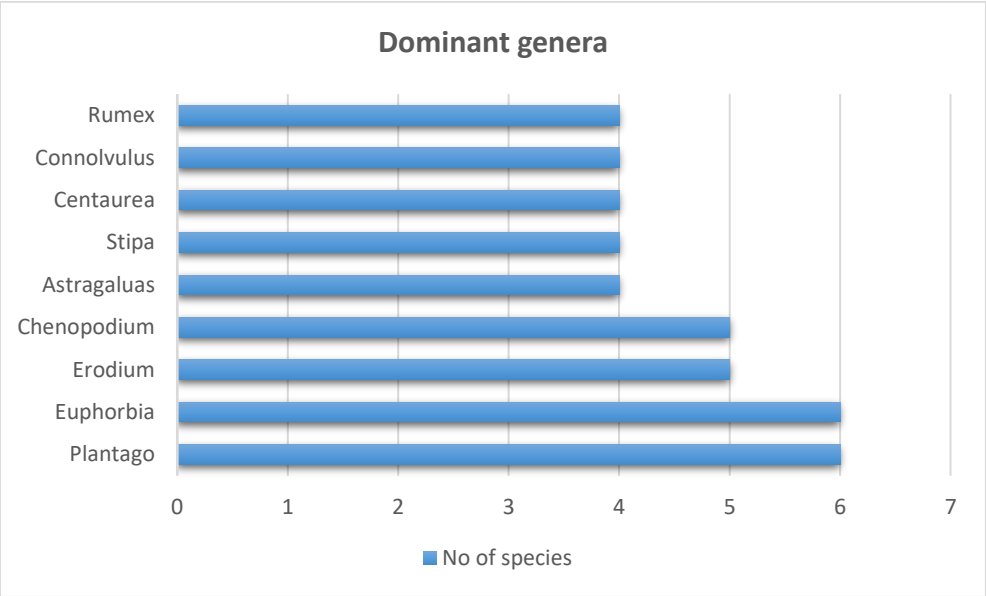


Figure 3: Dominant genera with their numbers.

Floristic list also presented in this study which provides us life forms and chorotypes of collected species. The status of each plant species according to the survey also indicated in the floristic list (Fig 4, Apendix). Analysis of Biological spectrum of collected plant species according to Raunkiaer system of life forms of plants, 1934 (Archibold, 1995) showed a predominance of Therophytes which comprise (61.2 %) with the number of (189) species, followed by Hemicryptophytes, which comprise (15.2 %) with the number of (47) species, then the Geophytes which comprise (15.2 %) with the number of (40) species. The rest of the result shown in the (Fig 4). As expected Therophytes have greater capacity for growth than other life forms, apparently because of their wider ecological amplitude, greater plasticity in size, and their small growth requirements. In addition, according to the result in (Fig 4), there is a clear positive correlation between Therophytes and Mediterranean chorotype, this explain why Therophytes dominating the study area which falls within the Mediterranean region.

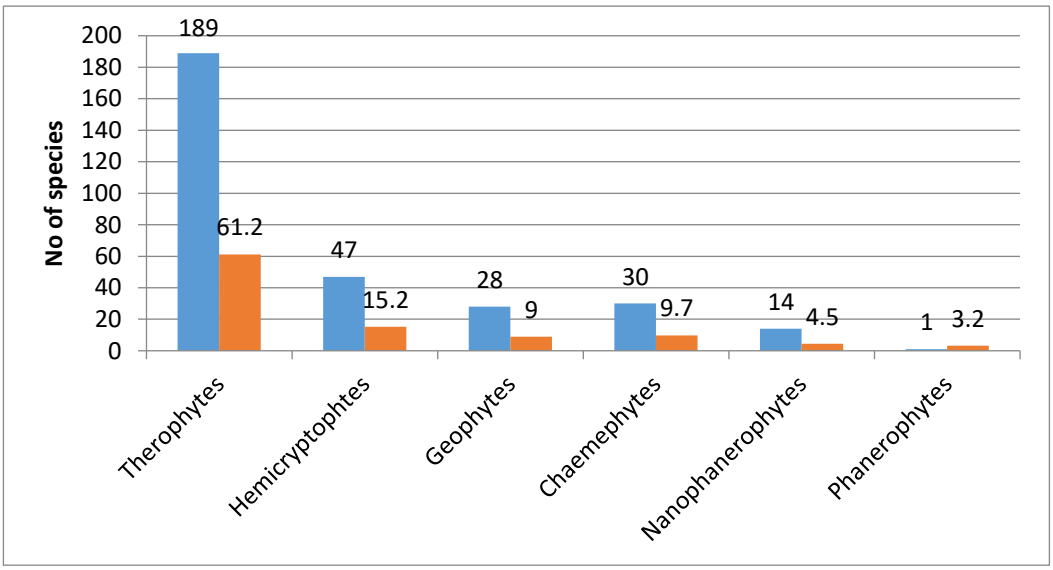


Figure 4: Lifeforms with their numbers and percentages.

Analysis of chorological spectrum of collected plant species showed a predominance of Mediterranean chorotypes which comprise (41.6. %) with the number of (128) species, followed by Mediterranean / Irano-Turanean chorotypes, which comprise (21.4 %) with the number of (66) species. (Fig 5).

This result is expected and not surprising because the study area is located mainly in the Mediterranean region which characterized by sub humid bioclimate, where the temperature is not very high and the moisture remained longer. The presence of Mediterranean / Irano-Turanean chorotypes with respected ratio because the Iranu-turanean region is overlapped with the east Mediterranean and both with more or less similar climate conditions, instead other chorological types were poorly represented, this may have been due to having been transported or introduced, or because the study area crossed by different wadies and characterized by different slope angles and different altitudes, this may cause the area characterized by different bioclimatic conditions leads to appearance of different chorological types.

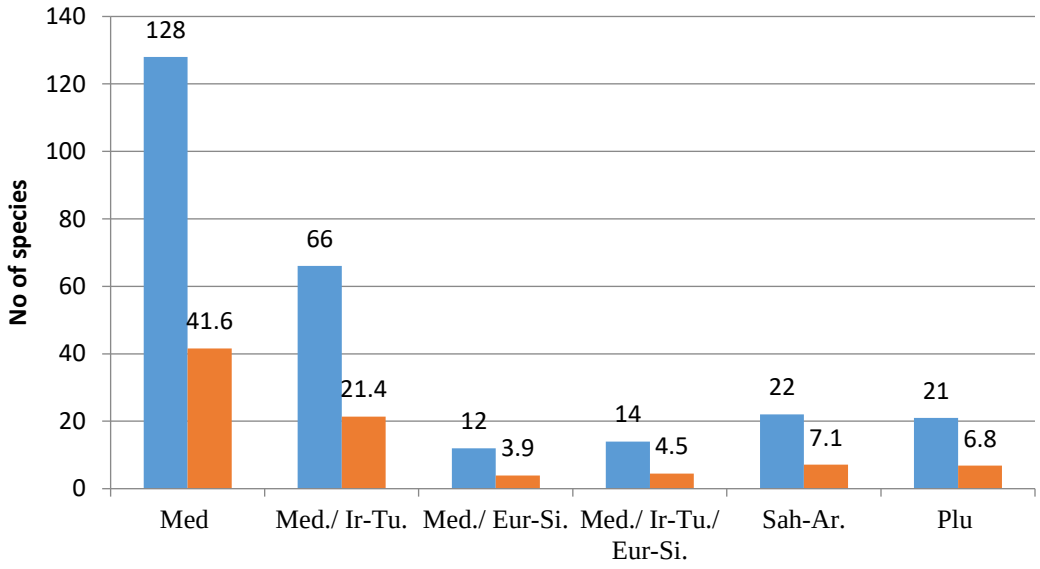


Figure 5: Chorotypes with their numbers and percentages.

4 Conclusions

The findings of this study have important implications for the understanding of plant diversity and distribution in the Al-Orban area of Gharyan district, Libya. The study provides a comprehensive inventory of plant species in the area and sheds light on the relative abundance of different plant families and genera. This information can be used to inform conservation efforts and land management strategies in the region. Furthermore, the study contributes to the broader understanding of plant biodiversity in Libya and provides a valuable resource for future research in this field.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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APPENDIX 1

Check List of Collected plant species of the study area

No	Family	Species	Lifeform	Chorotype
		Monocotyledons		
1	Alliaceae	*Allium ampeloprasum* L.	Geo	Med.
2	Alliaceae	*Allium oriental* Boiss	Geo	Med.
3	Alliaceae	*Allium roseum* L.	Geo	Med.
4	Iridaceae	*Gladiolus byzantinus* Miller.	Geo	Med.
5	Iridaceae	*Gladiolus segetum* Ker.- Gawl.	Geo	Med./ Ir-Tu.
6	Iridaceae	*Iris sisyrinchium* L.	Geo	Med.
7	Liliaceae	*Asparagus stipularis* Forsk.	Geo	Med.
8	Liliaceae	*Asphodelus tenuifolius Lav*	Geo.	Med./ Ir-Tu.
9	Liliaceae	*Asphodelus microcarpus* Salzm.&Viv.	Geo	Med.
10	Liliaceae	*Bellevalia sessiliflora* (Viv.)Kunth.	Geo	Med.
11	Liliaceae	*Colchicum ritchii* R.Br.	Geo	Med.
12	Liliaceae	*Dipcadi serotinum* (L.) Medic.	Geo	Plu.
13	Liliaceae	*Muscari comosum* (L.) Mill.	Geo	Plu.
14	Liliaceae	*Ornithogalum arabicum.*L.	Geo	Plu.
15	Poaceae	*Aegilops kotschyi* Boiss.	Th	Med./ Ir-Tu.
16	Poaceae	*Avena barbata* Pott exLink.	Th	Med./ Ir-Tu.
17	Poaceae	*Avena fatua* L.	Th	Med./ Ir-Tu./ Eur-Si.
18	Poaceae	*Avena sterilis* L.	Th	Med./ Ir-Tu.
19	Poaceae	*Bromus diandrus* Roth.	Th	Med.
20	Poaceae	*Bromus rigidus* Roth.	Th	Med./ Eur-Si
21	Poaceae	*Cenchrus ciliaris* L.	Th	Sah-Ar.
22	Poaceae	*Cutandia memphitica* (Sprengel.)Rich.	Th	Med./ Ir-Tu.
23	Poaceae	*Cynodon dactylon* (L.) Pers.	Geo	Boreal-Trop.
24	Poaceae	*Dactylis glomerata* L.	Th	Med./ Ir-Tu.
25	Poaceae	*Dactyloctenum aegyptium* (L.)Asch. & Cshw.	Th	Trop.
26	Poaceae	*Eleusine indica* (L.) Gaertn.	Th	Eur-Si.
27	Poaceae	*Hordeum murinum* Huds. subsp. *glaucum*	Th	Plu.
28	Poaceae	*Hyparrhenia hirta* (L.) Stapf.	H	Plu.
29	Poaceae	*Lagurus ovatus* L.	Th	Plu.
30	Poaceae	*Lamarckia aurea* (L.) Moench.	Th	Med./ Ir-Tu./ Sud.
31	Poaceae	*Lolium rigidum* Gaud.	Th	Plu.
32	Poaceae	*Lophochloa salzmannii* Boiss & H.Scholz.	Th	Med.
33	Poaceae	*Lygeum spartum* Loefl.ex Linn.	Geo	Med.
34	Poaceae	*Pennisetum setaceum* (Forsk.) Chiov.	Geo	Med./ Ir-Tu./ Sud.

No	Family	Species	Lifeform	Chorotype
35	Poaceae	*Phalaris minor* Retz.	Th	Med./ Ir-Tu.
36	Poaceae	*Phragmites australis* (Cav.) TrinexSteud.	Geo	Cos.
37	Poaceae	*Piptatherum miliaceum* (L.) Coss.	H	Med.
38	Poaceae	*Poa bulbosa* L.	Geo	Med./ Ir-Tu./ Eur-Si.
39	Poaceae	*Polypogon monspeliensis* (L.) Desf.	Th	Plu.
40	Poaceae	*Psilurus incurvus (Gouan.)* Schinz et Thell.	Th	Med./ Ir-Tu.
41	Poaceae	*Setaria adhaerens* (Forsk.) Chiov	Th	Plu.
42	Poaceae	*Stipa barbata* Desf.	Geo	Med./ Ir-Tu.
43	Poaceae	*Stipa capensis* Thunb.	Th	Med./ Ir-Tu./ Sah-Ar.
44	Poaceae	*Stipa parviflora* Desf	Geo	Med./ Ir-Tu.
45	Poaceae	*Stipa tenacessima* L.	Geo	Med.
46	Poaceae	*Stipagrostis ciliata* (Desf.) DeWinter.	Th	Med./ Ir-Tu.
47	Poaceae	*Trachynia distachya* (L.) Link.	Th	Med./ Ir-Tu.
Dicotyledons				
48	Amaranthaceae	*Amaranthus hybridus* L .	Th	Trop.
49	Amaranthaceae	*Amaranthus retroflexus* L.	Th	Med./ Eur-Si
50	Amaranthaceae	*Amaranthus viridis* L.	Th	Trop.
51	Anacardiaceae	*Pistacia atlantica* Desf.	NP	Med.
52	Anacardiaceae	*Rhus tripartita* (Ucria.) Grande.	NP	Ir-Tu.
53	Apiaceae	*Ammi visnaga* (L.) Lam.	Th	Med.
54	Apiaceae	*Apium graveolens* L.	Th	Med./ Ir-Tu.
55	Apiaceae	*Bunium fontainesii* (Pers) Maire.	Geo	Med.
56	Apiaceae	*Bupleurm lancifoluim* Hornem.	Th	Med./ Ir-Tu.
57	Apiaceae	*Bupleurm semicompositum* L.	Th	Med./ Ir-Tu.
58	Apiaceae	*Conium maculatum* L.	Geo	Med./ Eur-Si.
59	Apiaceae	*Daucus capillifolius* Gilli.	Th	Med.
60	Apiaceae	*Daucus syrticus* Murb.	Th	Med.
61	Apiaceae	*Ferula tingitana* L.	Th	Med.
62	Apiaceae	*Malabaila suaveolens* (Del.) Coss.	H	Med.
63	Apiaceae	*Pituranthos denudatus* Viv. Subsp. *battendieri*	Ch	Sah-Ar.
64	Apiaceae	*Smyrnium olusatrum* L.	H	Med./ Eur-Si.
65	Apiaceae	*Scandix pecten-veneris* L.	Th	Med./ Eur-Si.
66	Apiaceae	*Torilis leptophylla* (L.) Gaertn.	Th	Med./ Ir-Tu.
67	Apiaceae	*Torilis nodosa* (L.) Gaertn.	Th	Med./ Ir-Tu./ Eur-Si.
68	Apiaceae	*Torilis tenella* (Del.) Reichb.	Th	Med.
69	Asclepiadaceae	*Caralluma europaea* (Guss.) N.E.Br.	NP	Sud./ Sah-Ar.
70	Asclepiadaceae	*Periploca angustifolia* Labill.	NP	Med.
71	Asteraceae	*Anacyclus clavatus* (Desf.) Pers.	Th	Med.
72	Asteraceae	*Anacyclus monanthos* (L.)Thell.	Th	Med.
73	Asteraceae	*Anthemis secundiramea* Biv.	Th	Med.

No	Family	Species	Lifeform	Chorotype
74	Asteraceae	*Andryala integrifolia* L.	Th	Med.
75	Asteraceae	*Anvillea garcini* (Burm.fil.) DC.	Ch	Sah-Ar.
76	Asteraceae	*Artemisa monosperma* Delile.	Ch	Sah-Ar.
77	Asteraceae	*Artemisia herba-alba* Asso.	Ch	Ir-Tu.
78	Asteraceae	*Artemisia campestris* L.	Ch	Med./ Eur-Si.
79	Asteraceae	*Asteriscus pygmaeus*(DC.) Cosson & Durieu.	Th	Ir-Tu./ Sah-Ar.
80	Asteraceae	*Atractylis carduus* (Forsk.) Christensen.	H	Sah-Ar.
81	Asteraceae	*Atractylis cancellata* L.	Th	Med.
82	Asteraceae	*Atractylis delicatula* Batt. ex Chevall.	Th	Med.
83	Asteraceae	*Atractylis serratuloides* Bomel.	H	Sah-Ar.
84	Asteraceae	*Bombycilaena discolor* (Pers.) Lainz.	Th	Med.
85	Asteraceae	*Calendula arvensis* L.	Th	Med./ Ir-Tu.
86	Asteraceae	*Calendula tripterocarpa* Rupr.	Th	Sah-Ar.
87	Asteraceae	*Carduncellus eriocephalus* Boiss.	H	Sah-Ar.
88	Asteraceae	*Carduncellus pinnatus* (Desf.) DC.	H	Med.
89	Asteraceae	*Carduus getulus* Pomel.	Th	Sah-Ar.
90	Asteraceae	*Carthamus lanatus* L.	Th	Med./ Ir-Tu./ Eur-Si.
91	Asteraceae	*Centaurea dimorpha* Viv.	H	Med./ Ir-Tu.
92	Asteraceae	*Centaurea glomerata* Vahl.	Th	Med.
93	Asteraceae	*Centaurea maroccana* Boiss.	Th	Med.
94	Asteraceae	*Centaurea alexandrina* Delile.	Th	Med.
95	Asteraceae	*Chamomilla aurea* Loefl.	Th	Med./ Ir-Tu.
96	Asteraceae	*Chrysanthemum segetum* L.	Th	Med.
97	Asteraceae	*Conyza bonariensis* (L.) Cornq.	Th	Med.
98	Asteraceae	*Crepis libyca* (Pamp.) Shabet.	H	Med.
99	Asteraceae	*Crupina crupinastrum* (Moris.) Vis.	Th	Med./ Ir-Tu.
100	Asteraceae	*Cynara cardunculus* L.	H	Med.
101	Asteraceae	*Echinops galalensis* Schweinf.	H	Med.
102	Asteraceae	*Evax libyaca* Alavi.	Th	Med. (Endemic)
103	Asteraceae	*Filago desertorum* Pomel.	Th	Ir-Tu./ Sah-Ar.
104	Asteraceae	*Hedypnois cretica* (L.) Dum-Courset.	Th	Med.
105	Asteraceae	*Helianthus annuus* L.	Th	American
106	Asteraceae	*Helichrysum stoechas* (L.)Moench.	Th	Med.
107	Asteraceae	*Hyoseris scabra* L.	Th	Med.
108	Asteraceae	*Hypochoeris achyrophorus* L.	Th	Med.
109	Asteraceae	*Hypochoeris glabra* L.	Th	Med.
110	Asteraceae	*Jasonia rupestris* Bomel.	H	Med.
111	Asteraceae	*Koelpinia linearis* Pallas.	Th	Med./ Eur-Si.
112	Asteraceae	*Lactuca saligna* L.	Th	Med.
113	Asteraceae	*Launaea capitata* (Sprengel.) Dandy.	H	Sah-Sind.

No	Family	Species	Lifeform	Chorotype
114	Asteraceae	*Launaea nudicaulis* L.	H	Sah-Ar./ Sud. /Ir-Tu.
115	Asteraceae	*Launaea resedifolia* (L.). O.Kuntze.	H	Med.
116	Asteraceae	*Leontodon simplex* (Viv.) Widder.	Th	Med./ Eur-Si.
117	Asteraceae	*Nolletia chrysocomides* (Desf.) Cass.	H	Med.
118	Asteraceae	*Onopordum espinae* Cosson ex Bonnet.	H	Med.
119	Asteraceae	*Pallenis spinosa* (L) Cass.	H	Med.
120	Asteraceae	*Phagnalon rupestre* (L.) DC.	Ch	Med./ Ir-Tu.
121	Asteraceae	*Picris asplenoides* L.	Th	Sah-Ar.
122	Asteraceae	*Reichardia tingitana* (L.) Rorh.	Th	Ir-Tu./ Sah-Ar.
123	Asteraceae	*Rhagadiolus stellatus* (L.) Gaertner.	Th	Med./ Ir-Tu.
124	Asteraceae	*Scorzonera undulata* Vahl.	Geo	Med.
125	Asteraceae	*Senecio gallicus* Chiax	Th	Med.
126	Asteraceae	*Silybum marianium* Moench.	Th	Med./ Ir-Tu./ Eur-Si
127	Asteraceae	*Sonchus oleraceus* L.	Th	Cos.
128	Asteraceae	*Tripleurospermum fuscatum* (Desf.) Schultz.	Th	Med.
129	Asteraceae	*Urospemum dalechampii* L.	H	Med.
130	Asteraceae	*Xanthium spinosum* L.	Th	Boreal-Trop.
131	Boraginaceae	*Alkanna tinctoria* (L.) Tausch. subsp.*tripolitana* (Bornm) Jafri.	H	Med.
132	Boraginaceae	*Asperugo procumbens* L.	Th	Plu.
133	Boraginaceae	*Cynogolossum cheirifolium* L.	Th	Med.
134	Boraginaceae	*Echiochilon fruticosum* Desf.	Ch	Med.
135	Boraginaceae	*Echium angustifolium* Mill.	H	Med.
136	Boraginaceae	*Echium sabulicola* Pomel.	Th	Med.
137	Boraginaceae	*Elizaldia calycina* (Roem.&Schultes.) Maire.	Th	Med.
138	Boraginaceae	*Gastrocotyle hispida* (Forsk.) Bunge.	Th	Med./ Ir-Tu./ Sah-Ar.
139	Boraginaceae	*Heliotropium europaeum* L.	Th	Med.
140	Boraginaceae	*Nonea viviani* DC.	Th	Med. (Endemic)
141	Brassicaceae	*Alyssum montanum* L.	H	Med.
142	Brassicaceae	*Biscutella didyma* L.	Th	Med./ Ir-Tu.
143	Brassicaceae	*Brassica tournefortii* Gouan.	Th	Med./ Sah-Ar.
144	Brassicaceae	*Cardaria draba* (L.) Desv.	Th	Med./ Eur-Si.
145	Brassicaceae	*Carrichtera annua* (L.) DC.	Th	Med./ Ir-Tu./ Eur-Si.
146	Brassicaceae	*Clypeola jonthlaspi* L.	Th	Med./ Ir-Tu./ Eur-Si.
147	Brassicaceae	*Didesmus bipinnatus* (Desv.) DC.	Th	Med.
148	Brassicaceae	*Diplotaxis harra* (Forsk.) Boiss.	Th	Med./ Ir-Tu.
149	Brassicaceae	*Diplotaxis muralis* (L.) DC subsp.*muralis*	Th	Med./ Eur-Si.
150	Brassicaceae	*Enarthrocarpus clavatus* Del .ex Godr.	Th	Med.
151	Brassicaceae	*Eruca longirostris* Uechtr.	Th	Med.
152	Brassicaceae	*Eruca sativa* Mill.	Th	Med./ Ir-Tu.
153	Brassicaceae	*Farsetia aegyptia* Turra	Ch	Sah-Ar.

No	Family	Species	Lifeform	Chorotype
154	Brassicaceae	*Hussonia pinnata* (Viv.) Jafri.	H	Med.
155	Brassicaceae	*Lobularia libyca* (Viv.) Meisner.	Th	Med./ Ir-Tu.
156	Brassicaceae	*Lobularia maritima* (L.) Desv.	H	Med.
157	Brassicaceae	*Malcolmia africana* (L.) R.Br.	Th	Ir-Tu./ Sah-Ar.
158	Brassicaceae	*Matthiola longipetola* (Vent.) DC.	Th	Med./ Ir-Tu.
159	Brassicaceae	*Matthiola parviflora* (Schouboe.) R.Br.	Th	Sah-Ar.
160	Brassicaceae	*Matthiola tricuspidata* (L.) R.Br.	Th	Med.
161	Brassicaceae	*Neslia apiculata* Fisch.	Th	Med./ Ir-Tu.
162	Brassicaceae	*Notoceras bicorne* (Ait.) Caruel.	Th	Med.
163	Brassicaceae	*Sinapis alba* L.	Th	Med./ Ir-Tu./ Eur-Si.
164	Brassicaceae	*Sisymbrium erysimoides* Desf.	Th	Med./ Ir-Tu.
165	Brassicaceae	*Sisymbrium irio* L.	Th	Med./ Ir-Tu.
166	Caesalpiniaceae	*Ceratonia siliqua* Desf	Ph	Med.
167	Capparaceae	*Capparis spinosa* L	NP	Med.
168	Caryophyllaceae	*Dianthus crinitus* Sm.	H	Ir-Tu.
169	Caryophyllaceae	*Gypsophila pilosa* Huds.	Th	Ir-Tu.
170	Caryophyllaceae	*Minuartia geniculata* (Poiret.) Thell.	H	Med.
171	Caryophyllaceae	*Silene colorata* Poiret	Th	Med.
172	Caryophyllaceae	*Silene rubella* L.	Th	Med.
173	Caryophyllaceae	*Silene vulgaris* (Moench.) Garcke.	H	Eru-Si./ Med./ Ir-Tu.
174	Caryophyllaceae	*Spergula fallax* (Lowe) E.H.L.Krause.	H	Med./ Ir-Tu.
175	Caryophyllaceae	*Vaccaria pyramidata* Medik.	Th	Med.
176	Chenopodiaceae	*Anabasis articulata* (Forsk.) Moq. subsp. *oropediorum*	Ch	Sah-Ar.
177	Chenopodiaceae	*Arthrocnemum macrostachyum* (Moric) Moris.	Ch	Med./ Sah-Ar.
178	Chenopodiaceae	*Atriplex coriacea* Forsk.	Ch	Med./ Sah-Ar.
179	Chenopodiaceae	*Atriplex halimus* L.	NP	Med.
180	Chenopodiaceae	*Chenopodium ambrosioides* L.	Th	Plu.
181	Chenopodiaceae	*Chenopodium album* L.	Th	Plu.
182	Chenopodiaceae	*Chenopodium botrys* L.	Th	Plu.
183	Chenopodiaceae	*Chenopodium murale* L.	Th	Plu.
184	Chenopodiaceae	*Chenopodium vulvaria* L.	Th	Eru-Si./ Med./ Ir-Tu
185	Chenopodiaceae	*Hammada scoparia* (Pomel) Iljin.	Ch	Sah-Ar./ Ir-Tu.
186	Chenopodiaceae	*Kochia indica* Wight.	Th	Med./ Ir-Tu.
187	Chenopodiaceae	*Salsola kali* L.	Th	Plu.
188	Chenopodiaceae	*Salsola tetrarndra* Forsk.	Ch	Sah-Ar.
189	Chenopodiaceae	*Salsola vermiculata* L. var. *vermicutata*	Ch	Sah-Ar./ Ir-Tu.
190	Chenopodiaceae	*Suaeda aegyptiaca* (Hasselq.) Zoh.	Th	Sah-Ar.
191	Cistaceae	*Helianthemum ciliatum* (Desf.)Pers	Ch	Sah-Ar.
192	Cistaceae	*Helianthemum hirtum* (L.) Mill.	Ch	Med.
193	Convolvulaceae	*Convolvulus althaeoides* L.	Th	Med.

No	Family	Species	Lifeform	Chorotype
194	Convolvulaceae	*Convolvulus supinus* Coss. et. Kral.	Th	Med.
195	Convolvulaceae	*Convolvulus arvensis* L.	Geo	Plu.
196	Convolvulaceae	*Convolvulus oleifolius* Desr.	Ch	Med.
197	Crassulaceae	*Umbilicus horizontalis* (Guss.) DC.	H	Med.
198	Cucurbitaceae	*Citrullus colocynthis* (L.) Schrad.	H	Sah-Ar.
199	Cucurbitaceae	*Ecballium elaterium* (L.)A.Rich.	Th	Eru-Si./ Med.
200	Cuscutaceae	*Cuscuta planiflora* Ten.	Th	Med./ Ir-Tu.
201	Dipsacaceae	*Scabiosa arenaria* Forskal.	Th	Med.
202	Dipsacaceae	*Scabiosa monspeliensis* Jacq.	Th	Med.
203	Euphorbiaceae	*Chrozophora obliqua* (Vahi.) Juss.	Th	Med./ Ir-Tu.
204	Euphorbiaceae	*Euphorbia chamaesyce* L.	Th	Med./ Ir-Tu.
205	Euphorbiaceae	*Euphorbia falcata* L.	Th	Trop.
206	Euphorbiaceae	*Euphorbia helioscopia* L.	Th	Plu.
207	Euphorbiaceae	*Euphorbia parvula* Del.	Th	Med.
208	Euphorbiaceae	*Euphorbia retusa* Cav.	Th	Sah-Ar.
209	Euphorbiaceae	*Euphorbia serrata* L.	H	Med.
210	Euphorbiaceae	*Mercurialis annua* L.	Th	Med.
211	Euphorbiaceae	*Ricinus communis* L.	NP	Sud.
212	Fabaceae	*Anthyllis henoniana* Coss. ex Batt.	Ch	Med.
213	Fabaceae	*Astragalus hamosus* L.	Th	Med.
214	Fabaceae	*Astragalus sinaicus* Boiss.	Th	Med./ Ir-Tu.
215	Fabaceae	*Astragalus tribuloides* Del.	Th	Med./ Ir-Tu.
216	Fabaceae	*Calicotome villosa* (Poir.) Link.	NP	Med.
217	Fabaceae	*Coronilla scorpioides* (L.) Koch.	Th	Med.
218	Fabaceae	*Ebenus pinnata* Ait.	H	Med.
219	Fabaceae	*Genista microcephala* Coss & Dur.	NP	Med.
220	Fabaceae	*Hedysarum spinosissimum* L.	Th	Med.
221	Fabaceae	*Hippocrepis ciliata* Willd.	Th	Med.
222	Fabaceae	*Hippocrepis multisiliquosa* L.	Th	Med.
223	Fabaceae	*Hymenocarpos circinatus* L.	Th	Med./ Ir-Tu.
224	Fabaceae	*Lathyrus cicera* L.	Th	Med./ Ir-Tu.
225	Fabaceae	*Lotus edulis* L.	Th	Med.
226	Fabaceae	*Lotus glinoides* Del.	Th	Sud.
227	Fabaceae	*Medicago minima* (L.) Bart.	Th	Med./ Ir-Tu.
228	Fabaceae	*Medicago turbinata* (L.) All.	Th	Med.
229	Fabaceae	*Medicago polymorpha* L.	Th	Med./ Ir-Tu.
230	Fabaceae	*Melilotus sulcatus* Desf.	Th	Med.
231	Fabaceae	*Onobrychis caput- galli* (L.) Lam.	Th	Med.
232	Fabaceae	*Ononis reclinata* L.	Th	Med./ Ir-Tu.
233	Fabaceae	*Ononis viscosa* L.	Th	Med.

No	Family	Species	Lifeform	Chorotype
234	Fabaceae	*Psoralea bituminosa* L.	H	Med.
235	Fabaceae	*Retama raetam* (Forsk.) Webb.	NP	Sah-Ar.
236	Fabaceae	*Scorpiurus muricatus* L.	Th	Med.
237	Fabaceae	*Trifolium arvensis* L.	Th	Eru-Si./ Med./ Ir-Tu.
238	Fabaceae	*Vicia monantha* Retz.	Th	Med.
239	Geraniaceae	*Erodium hirtum* (Forsk.) Will.	Th	Sah-Ar.
240	Geraniaceae	*Erodium cicutarium* (L.) L.Herit.	Th	Med.
241	Geraniaceae	*Erodium malacoides* (L.) L.Herit.	Th	Med./ Ir-Tu.
242	Geraniaceae	*Erodium moschatum* (L.) L.Herit.	Th	Med.
243	Geraniaceae	*Erodium neuradifolium* Delile.	Th	Med./ Ir-Tu.
244	Geraniaceae	*Geranium molle* L.	Th	Med./ Eur-Si
245	Illecebraceae	*Gymnocarps decander* Forsk.	Ch	Med./ Ir-Tu.
246	Illecebraceae	*Herniaria cinerea* DC.	Th	Med./ Ir-Tu.
247	Illecebraceae	*Herniaria fontanesii* J.Gay.	H	Med.
248	Illecebraceae	*Paronychia chlorothyrasa* Murb.	Ch	Med.
249	Illecebraceae	*Pteranthus dichotomus* Forsk.	Th	Med./ Ir-Tu.
250	Illecebraceae	*Sclerocephalus arabicus* Boiss.	Th	Med./ Ir-Tu.
251	Lamiaceae	*Ajuga iva* (L.) Schreber.	H	Med./ Ir-Tu.
252	Lamiaceae	*Lamium amplexicaule* L.	Th	Med.
253	Lamiaceae	*Lavandula multifida* L.	Ch	Med./ Ir-Tu.
254	Lamiaceae	*Marrubium alysson* L.	H	Med.
255	Lamiaceae	*Marrubium vulgare* L.	H	Med./ Ir-Tu.
256	Lamiaceae	*Micromeria nervosa* (D,Urv.)Benth.	Ch	Med.
257	Lamiaceae	*Prasium majus* L.	NP	Med.
258	Lamiaceae	*Rosmarinus officinalis* L.	Ch	Med.
259	Lamiaceae	*Salvia aegyptiaca* L.	Ch	Sah-Ar.
260	Lamiaceae	*Salvia verbenaca* L.	Th	Med./ Ir-Tu./ Eur-Si.
261	Lamiaceae	*Teucrium compactum* L.	Ch	Med.
262	Lamiaceae	*Teucrium fruticans* L.	H	Med.
263	Lamiaceae	*Teucrium polium* L.	Ch	Med./ Ir-Tu./ Eur-Si.
264	Lamiaceae	*Thymus algeriensis* Boiss. et Reut.	Ch	Med.
265	Lamiaceae	*Thymus capitatus* (L.) Hoffm.& Link.	Ch	Med.
266	Malvaceae	*Malva parviflora* L.	Th	Med./ Eur-Si.
267	Malvaceae	*Malva sylvestris* L.	H	Med./ Ir-Tu.
268	Oxalidaceae	*Oxalis articulata* Savig.	Geo	Plu.
269	Oxalidaceae	*Oxalis pes-carpae* L.	Geo	Plu.
270	Papaveraceae	*Glaucium corniculatum* (L.) Rud.	Th	Med./ Ir-Tu.
271	Papaveraceae	*Papaver hybridum* L.	Th	Med.
272	Papaveraceae	*Papaver rhoeas* L.	Th	Med./ Ir-Tu.
273	Plantaginaceae	*Plantago albicans* L.	H	Med./ Ir-Tu.

No	Family	Species	Lifeform	Chorotype
274	Plantaginaceae	*Plantago arenaria* Waldst. & Kit.	Th	Med./ Ir-Tu./ Eur-Si.
275	Plantaginaceae	*Plantago coronopus* L.	Th	Med./ Ir-Tu.
276	Plantaginaceae	*Plantago amplexicaulis* Cav.	Th	Med./ Ir-Tu.
277	Plantaginaceae	*Plantago lanceolata* L.	H	Med./ Ir-Tu./ Sah-Ar.
278	Plantaginaceae	*Plantago ovata* Forskal.	H	Med./ Ir-Tu.
279	Polygonaceae	*Polygonum equisetiforme* Sibth & Sm.	Ch	Plu.
280	Polygonaceae	*Rumex bucephalophorous* L.	Th	Med.
281	Polygonaceae	*Rumex pulcher* L.	H	Temp.
282	Polygonaceae	*Rumex tingitanus* L.	Th	Ir-Tu.
283	Polygonaceae	*Rumex vesicarius* L.	Th	Sah-Ar.
284	Primulaceae	*Anagallis arvensis* L.	Th	Med./ Ir-Tu./ Eur-Si.
285	Ranunculaceae	*Adonis dentata* Delile.	Th	Med./ Ir-Tu.
286	Ranunculaceae	*Delphinium halteratum* Sibth & Smith.	Th	Med.
287	Resedaceae	*Reseda alba* L.	Th	Med./ Ir-Tu./ Eur-Si.
288	Resedaceae	*Reseda arabica* Boiss.	Th	Med./ Ir-Tu.
289	Resedaceae	*Reseda lutea* L.	Th	Med./ Ir-Tu.
290	Rhamnaceae	*Ziziphus lotus* (L.) Lam	NP	Med./ Sud.
291	Rubiaceae	*Galium tricornutum* Dandy.	Th	Med.
292	Rubiaceae	*Galium aparine* L.	Th	Med.
293	Rutaceae	*Haplophyllum tuberculatum* (Forsk.) Juss.	H	Sah-Ar./ Sud.
294	Rutaceae	*Ruta chalepensis* L.	Th	Ir-Tu./ Sah-Ar.
295	Scrophulariaceae	*Kickxia aegyptica* L. subsp *fruticosa*	H	Med./ Sah-Ar.
296	Scrophulariaceae	*Linaria tarhunensis* Pamp.	Th	Med.
297	Scrophulariaceae	*Linaria virgata* (Poir.) Desf.	Th	Med.
298	Scrophulariaceae	*Scrophularia arguta* Aiton.	Th	Med./ Sah-Ar.
299	Solanaceae	*Datura innoxia* Mill.	Th	American
300	Solanaceae	*Hyoscyamus albus* L.	H	Med./ Ir-Tu.
301	Solanaceae	*Lycium europaeum* L.	NP	Med.
302	Solanaceae	*Nicotiana glauca* R.C.Graham.	NP	Plu.
303	Solanaceae	*Solanum nigrum* L.	Th	Cos.
304	Thymelaeceae	*Thymelaea hirsuta* (L.) Endl.	Ch	Med.
305	Urticaceae	*Urtica pilulifera* L.	Th	Med./ Ir-Tu./ Eur-Si
306	Urticaceae	*Urtica urens* L.	Th	Med./ Ir-Tu.
307	Valerianaceae	*Centranthus calcitrapae* (L.) Dufresne.	Th	Med.
308	Valerianaceae	*Valerianella discoidea* (L.) Loisel.	Th	Med./ Ir-Tu.
309	Zygophyllaceae	*Peganum harmala* L.	Th	Med./ Ir-Tu.

APPENDIX 2

Definitions of lifeforms

Chaemephytes	Subshrubs
Geophytes	Perennial herbs with bulbs, corms, tubers or rhizomes
Hemicryptophytes	Perennial herbs
Nanophanerophytes	Shrubs
Phanerophytes	Trees
Therophytes	Annual herbs

APPENDIX 3

Definitions of Chorotypes

Cos.	Cosmopolitan
Eur-Si.	Eurosiberian
Ir-Tu.	Iranutuanean
Med	Mediterranean
Plu.	Pluriregional
Sah-Ar.	Saharabian
Temp	Temperate
Trop.	Tropical

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Prediction and Analysis of Targeting Libyan Severe Acute Respiratory Syndrome Corona Virus 2 isolates by Micro-RNA

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A B S T R A C T

The COVID-19 pandemic has caused widespread concern, and extensive studies have been conducted to discover an effective therapy for the virus, some of these studies have demonstrated that host miRNAs have antiviral properties and may enhance the treatment of individuals with COVID-19. Host miRNAs are important regulators of virus replication and translation by binding directly to viral RNA. Investigating the interaction between miRNA and SARS-CoV2 can reveal novel therapeutic approaches against this virus. The study analyzed the genomes of seven Libyan SARS-CoV2 isolates and the Wuhan reference strain and used bioinformatics prediction to identify human mature miRNAs that interact with the virus. The study found that 142 lung miRNAs could interact with the viral RNA, and identified several miRNAs with multiple binding sites, including hsa-mir-197-5p and hsa-mir-286-3p. The study also identified miR-138-5p and miR-574-5p as potential therapeutic targets, as they have the ability to bind to the 3'UTR of IFN and ACE2 genes in the host cell. However, the interactions between miRNA and mRNA identified in this study require further experimental validation to confirm their therapeutic potential.

1 Introduction

SARS-CoV2 is an enveloped, positive-sense, single-stranded RNA virus that belongs to the family of coronaviruses. The virus primarily infects the respiratory tract and can cause severe respiratory illness ranging from mild cold-like symptoms to severe acute respiratory syndrome and death. COVID-19 has a high transmission rate and mortality rate, making it a significant threat to global public health. The outbreak of the novel coronavirus disease (COVID-19) caused by (SARS-CoV2) has rapidly spread worldwide, resulting in a global health crisis. Despite extensive efforts by the scientific community, there is currently no specific treatment or vaccine available for COVID-19 (Agarwal *et al.*, 2015). Therefore, there is an urgent need to identify potential therapeutic targets and develop effective treatments against SARS-CoV2. MicroRNAs (miRNAs) are small non-coding RNAs that play a crucial role in regulating gene expression in eukaryotes. Recent studies have shown that host miRNAs can regulate the replication and translation of viral RNA by directly binding to viral RNA (Discovery & Alam, 2021). Investigation into the interaction between miRNAs and SARS-CoV2 can reveal novel therapeutic approaches against this virus. MicroRNAs are small RNA molecules that regulate gene expression by binding to the 3' untranslated region (UTR) of target messenger RNAs (mRNAs), leading to their degradation or translational repression. Recent studies have shown that host miRNAs can also regulate viral replication and translation by direct binding to viral RNA in infected cells(Agarwal *et al.*, 2015).

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Bioinformatics prediction is a computational approach used to predict the interaction between two or more biological molecules, such as proteins, DNA, RNA, and miRNA. In the case of miRNA-virus interaction, bioinformatics prediction is used to identify potential binding sites between host miRNAs and viral RNA (Identify *et al*., 2020). The prediction is based on the sequence complementarity between miRNA and RNA, particularly in the seed region (positions 2-8) of the miRNA. The seed region is the most important region of miRNA for target recognition and binding, and it requires perfect or near-perfect complementarity with the target RNA to initiate the miRNA-mediated gene silencing process (Identify *et al*., 2020). There are several software programs and databases available for miRNA target prediction, including TargetScan, miRanda, and RNAhybrid, among others. These programs use algorithms to predict the most likely binding sites between miRNA and RNA based on sequence complementarity, thermodynamic stability, conservation, and other factors. However, it is important to note that bioinformatics prediction is not always accurate, and experimental validation is required to confirm the predicted miRNA-virus interactions.

Therefore, bioinformatics prediction should be considered as a preliminary step in identifying potential miRNA-virus interactions, and not as conclusive evidence of their existence(Chauhan *et al*., 2022; Nieto-d *et al*., 2021).

The genomic similarity between SARS-CoV2 and mRNAs suggests the presence of micro-RNA binding sites that could potentially reduce virus expression and translation. Given the lack of specific treatments available for COVID-19 patients, exploring the role of cellular miRNAs in SARS-CoV2 pathogenesis could lead to the development of promising therapeutic options.

Our study is one of the first conducted in Libya to investigate the interaction between human miRNAs and Libyan SARS-CoV2 isolates. However, current treatment options for SARS-CoV-2 remain limited in their safety and efficacy. In this study, we aimed to identify lung epithelial miRNAs that could potentially regulate virus replication and expression in Libyan SARS-CoV2 isolates, as well as host miRNAs that could regulate ACE2 and TMPRSS2.

2 Materials and Methods

2.1 Retrieving SARS-CoV2 Genome Sequences

Seven nucleotide sequences of Libyan SARS-CoV2 isolates were downloaded as FASTA files from National Center for Biotechnology Information (NCBI), with accession numbers (MW018435, MW018429, MW018431, MW018436, MW018428, MW018434, MW018432). These seven viral genomes were detected in Libyan SARS-CoV2 positive nasopharyngeal samples in June 2020 Virus strain isolated in Wuhan, China (NC-045512.2) was also retrieved and considered as the reference viral sequence in this study (Yao *et al*., 2019).

2.2 Obtaining Human miRNAs Sequences

All human mature miRNA sequences were accessed from the miRBase database version 22.1 (Siniscalchi *et al., 2021)*. This study only analyzed high-confidence mature miRNAs that have been reported to have target binding sites or have a role in viral host interaction in peer-reviewed original articles in previous studies.

2.3 MiRNAs Expression in Human Lung Tissue

Selected miRNAs were again filtered to analyze only high-confidence miRNAs that express in the lung epithelial cells which are the main target of the SARS-CoV2. Lung micro-RNA expression data were extracted from the Human miRNA Tissue Atlas (https:// ccb-web.cs.uni-saarland.de/tissueatlas). This is a web-based repository of experimental data collection by microarray analysis for the detection of 1997 microRNAs in 61 tissue biopsies of different human organs (Young *et al*., 2022).

The miRNA that was not found in Human miRNA Tissue Atlas database, was obtained from the Cancer Genome Atlas lung adenocarcinoma (TCGA-LUAD) project utilizing only the matched normal tissue specimen (Zealy *et al*., 2017), this database is available from the TCGA Research Network database: (https://portal.gdc.cancer.gov/projects/TCGA-LUDA)

2.4 RNA-RNA Interaction Analysis:

To find miRNAs that can bind to viral RNA, two freely available online tools were used as Insilco screening:

RNA Hybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid), using a cut off parameter for free energy of binding (mfe) ≤ -25kcl/mol (Wong & Saier, 2021), and 150 binding site

per RNApar, no other parameters were modified. The miRNA that binds with the viral genome with 100% seed site complementary was considered (the size of seed 7mer) from nucleotide 2 to nucleotide 8 in the 5` of miRNA sequence.

RNA22 Version 2 (https://cm.je_erson.edu/rna22/Interactive), this prediction tool used to identify microRNA region elements (MREs) in the viral genome to miRNAs that showed considered targets in the RNA hybrid tool results. Only significant miRNA- MRE prediction ($P<0.05$) with a seed size of 7 nucleotides and no unpaired base were registered (Wong & Saier, 2021).

3 Results

3.1 Libyan SARS-CoV$_2$ Isolates Have Numerous Human miRNAs Binding Sites

The miRBase database version 22.1 was used to identify human mature miRNAs with direct binding sites on different coronavirus isolates from different geographical regions worldwide (Table 1). Only 228 high confidence miRNAs were identified based on the criteria in miRBase. From these high confidence ones, 219 were found expressed in lung epithelial cells according to the human microRNA Atlas database and TCGA-LUAD project. The study focused on miRNAs showing interactions with perfect matching in the seed region with free energy $\Delta G \leq 25$kcal/mol. A total of 142 miRNAs were sorted out by RNA hybrid tool. According to the results, there were 38 miRNAs with more than three binding sites on the seven analyzed Libyan coronavirus 2 isolates. Among them, eight showed more than 7 target hits, namely: (hsa-mir-149-3p, hsa-mir-103-3p, hsa-mir-4676-5p, hsa-mir-143-5p, hsa-mir-3619-5p, hsa-mir-296-3p, hsa-mir-885-3p, hsa-mir-197-5p). Notably, the hsa-mir-197-5p and hsa-mir-286-3p revealed the greatest number of interactions (12 and 11 sites respectively) with SARS-CoV2 (table 2).

The position of binding sites on the analyzed SARS-CoV2 for high confidence lung miRNAs was also explored and it was noticed that the predicted targets occurred in various locations in the genome, including genes encoding structural proteins as well as non-structural proteins coding genes (Nsps). In this study, out of the four structural protein genes (E, M, N, S) in COVID-19 genome, S gene is the most targeted gene as it is the largest structural gene followed by N gene and then M gene. Only one high-confidence human miRNA (hsa-mir199a-3p) targets E gene.

Out of the 16 Nsps of SARS-CoV2, RNA hybrid tool predicted (most targets on Nsp3 and Nsp2 then Nsp4, Nsp6-10). No hits were predicted on Nsp1, Nsp5 and Nsps11-16; again, the numbers of targeting sites appeared to be correlated with the gene length.

Additionally, the predicted targets of the human miRNAs on viral genomes were not restricted to gene bodies; three miRNA (hsa-mir-4684-3p, hsa-mir-4786-3p and hsa-mir-3614-5p) were found targeting 5` untranslated region with ΔG -27, -30.1 and -30,1 respectively. No high confidence miRNA was predicted to target viral 3` UTR with 100% complementary in seed site region and $\Delta G \leq 25$ kcal/mol in this study. The target sequence identified by two predictive tools RNA hybrid and RNA22 were then filtered to consider only the position targets that were validated by both tools in all seven tested COVID strains. This selection yielded 11 high confidence lung miRNAs with more validated targets (table 3). Four targeted (ORF1ab), which encoding 5` viral replicase, one target spike protein which responsible for attachment to ACE2 receptor for host cell entry, one targeted (ORF7a) which encoding transmembrane protein involved in apoptosis induction and five miRNA targeted N gene which responsible for form viral nucleocapsid.

3.2 miRNAs Viral Genome Interaction and mRNA Entry Receptor Interaction

Given that viral entry is crucial for the infectivity and pathogenesis of SARS-CoV2, this research investigated the ability of predicted miRNAs (53 miRNAs) that have been reported to target the 3'UTR of host receptor proteins ACE2 and TMPRSS, as well as their upstream regulator IFN genes, to bind to SARS-CoV2 genomes.

Remarkably, the binding prediction tool revealed that hsa-mir-138-5p, which can bind to the 3'UTR of the INF-beta gene, has six binding sites on SARS-CoV2. Additionally, hsa-mir-574-5p, which binds to the 3'UTR of the ACE2 gene, has four binding sites in the viral genome.

Table (1): Description of Libyan SARS-CoV2 isolates that analyzed in this study

Isolates	Gene-Bank Accession number	Gender	Age	Origin	Date	Super spreader cluster
1	MW018435	F	50	Tripoli	04 June 2020	SS_1
2	MW018429	F	35	Tripoli	19 June 2020	SS_1
3	MW018431	F	40	Tripoli	15 June 2020	SS_1
4	MW018436	ND	ND	Sabha	ND	SS_1
5	MW018428	M	47	Tripoli	ND	SS_1
6	MW018434	M	4	Tripoli	ND	SS_1
7	MW018432	M	47	Tripoli	13 June 2020	SS_1

*F: Female, M: Male, ND: no data available.

Table (2): MiRNAs with more than 3 binding site on the four SARS-CoV2 genomes

N	miRNA ID	Number of binding sits	N	miRNA ID	Number of binding sits
1	Hsa-miR-199a-3p	5	20	Has-miR-365a-5p	6
2	Hsa-miR-93-5p	5	21	Has-miR-550a-5p	4
3	Hsa-miR-135a-3p	4	22	Has-miR-3065-3p	4
4	Hsa-miR-3619-5p	9	23	Has-miR-3130-3p	4
5	Hsa-miR-330-3p	5	24	Has-miR-4676-5p	8
6	Hsa-miR-103a-3p	8	25	Has-miR-4687-5p	4
7	Hsa-miR-3189-3p	4	26	Has-miR-17-5P	5
8	Hsa-miR-17-3p	5	27	Has-miR-214-3p	4
9	Hsa-miR-3194-5p	5	28	Hsa-miR-197-5p	12
10	Hsa-miR-29c-3p	5	29	Has-miR-5001-3p	7
11	Hsa-miR-4684-3p	4	30	Hsa-miR-494-5p	6
12	Hsa-miR-29a-3p	5	31	Has-miR-15b-5p	5
13	Hsa-miR-143-5p	9	32	Hsa-miR-323b-5p	7
14	Hsa-miR-4786-3p	6	33	Hsa-miR-296-3p	11
15	Hsa-miR-6834-5p	4	34	Hsa-miR-323b-5p	7
16	Hsa-miR-760	5	35	Hsa-miR-296-3p	11
17	Hsa-miR-107	7	36	Hsa-miR-885-3p	11
18	Hsa-miR-574-5p	4	37	Hsa-miR-149-3p	8
19	Has-miR-29b-3p	5	38	Has-miR-138-5p	6

Table (3): MiRNAs that binds in the same position in the two prediction tools

Human miRNA	Target position (RNA hybrid)	Mfe And P-value	Lung repression copy per million	miRNA-mRNA paring
Has-miR-4676-5p	4762	-27.7 P = 0.05	20،31631	AAACCATCTCACTTGCTGGTTC || |||||: :||||:|| AGTGACAGAGTGG-TGACCGAG
Has-miR-4687-5p	440	-30.0 P = 0.03	16228،47	AACTGC-ACCTC--ATGGTCA |||| |||| |::|:|| CGGACGGGGGAGGTTGTCGGT
Has-miR-197-5p	3010	-31.7 P = 0.007	29739	ATATGTATTGTTCTTTCTACCCT : :|:||::||:|||||| GGAGGGTGACGGGAGAGATGGG C
Has-miR-138-5p	23100	-25.8 P = 0.05	3.23564	TGGAAAAACTCAAAACACTAGTT :|| ||| ||||:||: GCCGGACTAAGTGTTGTGGTCGA
Has-miR574-5p	27719	-25.1 P = 0.004	2494،975	TAACACTT-TGC-TTCACACTCA |||||: ::| ||||||| TGTGTGAGTGTGTGTGTGTGAGT
Hsa-miR-3189-3p	29099	-26.4 P = 0.05	7.56629	GTGGTCCAGAACAAACCCAAGGA |: :||| || ||||||| GATGGGGT--AGTCTGGGTTCCC
Hsa-miR-3619-5p	29440	-25.3 P = 0.01	3,82595	ACTGTTACTCTTCTTCCTGCTGC |||: ||: :|| |||||| CGACG-TGGTCGGACGGACGACT
Hsa-miR-514a-3p	2499	-26.6 P = 0.001	0,20335	ACTTCCCACAGAAGTGTTAAC || | |||||||||:|| AGATGAGTGTCTTCACAGTTA
Hsa-miR-502-5p	28436	26.3- P = 0.008	2,45825	AAATTCCCTCGAGGACAAGGCGT T ||| |: |||||:| TGCCAGGATGT--GAGTTCCGTAC
Hsa-miR-4684-3p	29476	-25.8 P = 0.05	4,00759	ATTTCTCCAAACAATTGCAACA ||||| ||||||| TGCAGAGGTGGCTGAACGTTGT
Hsa-miR-219a-1-3p	29506	26.1- P = 0.05	1,064817	ATGAGCAGTGCTGACTCAACTCA :| || | |||||||| GCCCTG-CAGGTCTGAGTTGAGA

4 Discussion

The potential role of miRNAs in regulating host-virus interactions during infections. Host cells can use miRNAs to limit viral replication by targeting viral genomes or transcripts directly or indirectly by modulating host factors involved in the immune response. However, long-standing viral infections can lead to a balance between host and virus, where viruses adapt to coexist with host cells by utilizing mRNA machinery. For example, in HBV infection, the virus-encoded HBx protein up-regulates the expression of miR-125, which inhibits viral replication and allows the virus to evade immunoreceptors and keep host cells alive (Shimakami *et al*., 2012).

In the case of SARS-CoV2, several studies have predicted cellular miRNAs targeting the viral genome, but few have had experimental validation (Hardin & Xiao, 2022; Li *et al*., 2022). This study focused on high-confidence lung miRNAs interacting with Libyan SARS-CoV2 isolates using bioinformatics prediction. The study identified several miRNAs with multiple binding sites, suggesting that these miRNAs may be sequestered during SARS-CoV2 replication, leading to a reduction in their availability, which could disrupt normal pathways regulated by the mRNA (Li *et al*., 2022).

The study identified several miRNAs that may have potential therapeutic applications, including miR-197-5p, which has been associated with defense mechanisms against certain viruses and has been reported to play a vital role either as an oncogene or tumor suppressor in different cancers (Jain *et al*., 2019). MiR-138-5p was predicted to target the IFN beta and ACE genes, and it has experimental evidence of having an antiviral role during HIV infection and acting as a tumor suppressor gene for many types of cancers (Xu *et al*., 2020). Additionally, miR-138-5p targets the EZH2 gene, whose inhibition induces a potent antiviral state and suppresses infection by DNA and RNA viruses.

5 Conclusions

The study found that several lung miRNAs, including has-mir-138-5p and has-mir-197-5p, have the potential to interact with Libyan SARS-CoV2 genomes, with miR-138 also having a potential role in regulating ACE2 expression. However, further in-vitro and in-vivo experiments are needed to validate the potential antiviral activity of these miRNAs.

Disclaimer: The article has not been previously presented or published and is not part of a thesis project.

Conflict of Interest: There are no financial, personal, or professional conflicts of interest to declare.

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Isolated Some Fish Parasites from *Sarpa salpa* (Linnaeus, 1758) in Misurata City Coast, Libya

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A B S T R A C T

In this study, some parasitic infections were investigated in a type of herbivorous marine fish that is widely spread in the coastal water of Misurata city. The abdominal cavity, gills, stomach, intestines, liver, and spleen of ten *Sarpa salpa* fish were examined between February and April 2018. The results showed that the incidence of helminths varied among the study fish, with the highest infection rate recorded in the posterior and interior intestines, reaching 31-30% respectively, followed by the stomach at a rate of 22%. The liver had a parasitic infection rate of 17%, specifically Trematoda. Acanthocephalan was only observed in the posterior intestines. While the gills, abdominal cavity, spleen, and ectoparasites, their infection rates were 0%. Microscopic examination of the liver, gills, and intestines' tissues revealed the presence of various tissue diseases, including swelling, cysts, and degradation of most liver cells, as well as damage to the gill filaments.

1 Introduction

Fishes are considered one of the most important sources rich in protein, providing 16% of animal protein, vitamins, and essential minerals (Kassem and Bowashi, 2015). Therefore, fishery resources have become among the sources that many countries increasingly rely on to fill the food gap, and many studies have been conducted to identify the sources of pollution that affect fish in their natural environment (Bastawrows, 2003). Fishes are carriers of a large number of parasites, and thus the pathogenic effect of parasites that infect fish varies with each parasite, some of which cause little harm, while others can be severe and lead to death (Overstreet and Hawkins, 2017). Many parasites that infect fish cause the host to be deprived of part of its food, which affects its growth. Also, the various activities of fish are affected as a result of their activity within the host's tissues or secretion of some substances that may be toxic, reducing the nutritional value of fish. Fishes play a carrier or transporter role for other pathogens, making some of them a source of disease for other fish or vertebrates, including humans, where they are transmitted by consuming raw or poorly cooked fish (Al-Alusi, 2011; Kassem and Bowashi, 2015). Parasitic diseases make up about 80% of all diseases that affect marine vertebrates, where warm-water fish with stable temperatures throughout the year are found, and this climate helps provide natural food in the water and the proliferation of intermediate hosts such as snails, copepods, crustaceans, Leechesand barnacles. Parasites are the primary source of most pandemic and epidemic diseases that enter as secondary infections like bacteria and fungi, which leads to significant economic losses due to high infection and mortality rates (Marcogliese, 2008).

Infection with helminths parasites such as Trematoda, Nematoda, and Acanthocephalan are among the most common parasites that affect fish, and the digestive

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system and its accessories are considered among the organs susceptible to infection by these parasites, by ingestion of food contaminated by Infective stages or ingestion of animal tissues representing Intermediate hosts for some helminths (Al-Saadi *et al.*, 2016). Some fishes are considered intermediate hosts for Trematoda such as *Heterophyes heterophyes*, which cause some intestinal disorders in humans and stunted growth in children,and are considered one of the most dangerous helminthes for humans (Bastawrows, 2003). Acanthocephalan are common intestinal parasites, especially in fish intestines, and have an intermediate host that is represented by arthropods such as crustaceans (Taher *et al.*, 2017). Trematoda has a complex life cycle that requires more than one host, and most of them are hermaphroditic. Trematoda is a common helminths all classes of vertebrates, especially marine fish, and ranks second after Nematoda in their distribution, where the mature parasite produces eggs that pass through the final host's feces. Thousands of species of Trematoda have been described in different types of fish all over the world. The clinical picture of infection with these parasites depends on the size and number of helminths present in the host, organs, or affected tissues. The infection may be local or systemic, usually both, resulting in tissue ulceration and abscess formation. In fish, adult Trematoda are mostly found in the intestines and stomach, and some have been found in the liver, bile ducts, and other organs. Fish can act as final, intermediate, or carrier hosts or reservoirs for different species of Trematoda. It is also known that some types of animal infections caused by Trematoda affect humans and cause diseases, and many species cause economic losses to society (Shaukat., 2008).

A study found the presence of Trematoda infection in two species of marine fish, *Abudefduf luridus* and *Chromis limbata*, while no infection was recorded in *Boops boops* (Costa and Biscoito, 2003). Another study conducted on *Selene setapinnis* fish showed their infection with different types of Trematoda (Cordeiro and Luque, 2004).

As indicated by Parveen *et al.*, (2018) in their study on a type of marine fish, *Plectorhinchus cinctus*, infections with the parasite Trematoda were recorded in the stomach, leading to tissue damage. Similarly, Pardeshi *et al*., (2012) explained in their study on tissue infections in a type of marine fish that the infections were due to the parasite Trematoda, resulting in abscesses, changes in the overall shape of the liver, and tissue damage. In a study by Abdul-Ameer (2010), two types of Trematoda were recorded in the gills of *Aspius vorax* and *Barbus sharpeyi*. As mentioned by Heckmann (2001) in his study on fish species, the parasite Acanthocephalan was found in many organs of the study fish, including the liver, spleen, and muscles. AL-Zubaidy *et al*., (2012) isolated five species of the parasite Acanthocephalan from 11 species of marine fish, where the study indicated the spread of adult Acanthocephala parasites in the intestinal cavity, while the larval stage was present in the body cavity and some organs such as the liver and spleen. Also, the study conducted by Alabbar (2014) on *Muraena helena*. showed the presence of three types of helminths on the internal parts of the fish (*Anisakis* sp., *Cucullanus* sp., Nematodes sp.), with infection rates of 96.49%, 57.02%, and 26.32% respectively.

Improving fish production in natural water and increasing it requires conducting many studies and research related to the aspects that negatively and positively affect fish productivity. Due to the importance of this topic, the current study was conducted and it was found necessary to provide effective solutions against helminths that infect some species of economically important fish on the coast of Misurata city and surrounding areas. This is a new addition to previous studies and research on fish parasites on the Libyan coast.

2 Materials and Methods

The targeted fish for this study were collected from the fishing port in Qasr Ahmed in the city of Misurata, Libya as shown in Figure (1), during the period between February and April 2018. The collection included 10 herbivorous fish of the *Sarpa salpa* species (Figure 2), with 4 collections per month during the study period, using trawl nets for fishing.

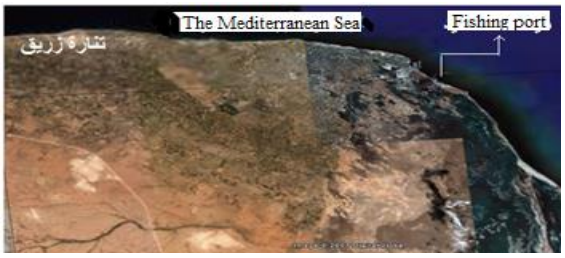

Figure (1): The location of the collection of study samples.

Figure (2): External feature of *S. salpa*. (TL): Total length.

The fish were transferred directly to the laboratory at the faculty of science at Misurata University in individual bags. External morphometric measurements were taken for the fish, where the average total length of *S. salpa* fish ranged between 30.04±0.75 and the average weight ranged between 343.80±9.27. The fish were examined directly (With the naked eye) to detect any external observations or changes that may indicate infection with parasites, noting any changes in the color of the fish or eye color. The fish were dissected and then the abdominal cavity and digestive tract were examined after a longitudinal incision was made at the median abdominal line, in addition to examining the gills after removing the fish's operculum. The digestive tract was separated from the body, and the intestines were opened along their length inside a Petri dish containing a physiological solution and examined visually for helminths inside them. Similarly, the gills, stomach, liver, and spleen were examined, according to what DE Giusti (1949) mentioned.

The large parasites were transferred to containers containing 5% formalin and tightly sealed, with the type of fish (Crites and Overstreet, 1991) written on them. Some of them were also placed in an ethanolic solution for ten minutes, spread on glass slides, and covered with a cover slide for examination under a light microscope, as reported by Al-Taee, and Zangana (2011).

Parts of different areas of fish samples, such as gills, digestive tract (1cm), and liver, were taken and placed in 10% formalin for histological examination after sections were taken using a microtome, according to Sheehan and Hrapchak (1980).

In addition to determining the density of infection for each parasite, some mathematical equations were used based on the study presented by Margolis *et al.,* (1982) to the American Society of Parasitologists regarding the adoption of infection standards according to infection terms, as reported by Al-Alusi, (2011) and Al-Saadi., *et al* (2016), as follows:

$$\text{Infection Rate} = \frac{Number\ of\ infections\ in\ the\ month}{Total\ number\ of\ samples\ in\ the\ month} \text{x}100$$

$$\text{Intensity of infection rate} = \frac{Number\ of\ isolated\ worms}{Number\ of\ infected\ fish}$$

2.1 Statistical Analysis

Statistical analysis was conducted using the statistical software SPSS (Version 25) to find the relationship between the number of parasites and their location in fish, as well as some descriptive measures including mean, standard error, and percentage of all samples.

3 Results

The results showed the presence of different parasitic infections with some helminths, where the total infection rate and the monthly infection rate in the *S. salpa* fish during the study months were shown in Table (1).

Table (1): shows the total infection rate and the monthly infection rate during the study months.

Study's duration	Number of samples per month	Overall infection rate %
February 2018	3	30%
March 2018	3	30%
April 2018	4	40%
Total	10	100%

The current study results show that no external helminths parasites were found during the external examination of *S. salpa* fish, as shown in Figure (3)

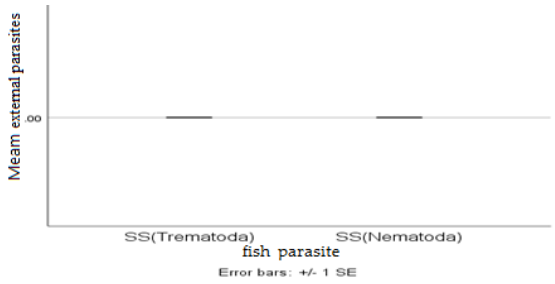

Figure (3): Ectoparasites of (SS): *S. salpa*.

Similarly, examination of the gills of the studied fish did not reveal any infection with Acanthocephalan or Trematoda (Figure 4). Microscopic examination of the abdominal cavity of *S. salpa* fish revealed no infection with any Acanthocephalan and Trematoda parasites, as shown in Figure (5).

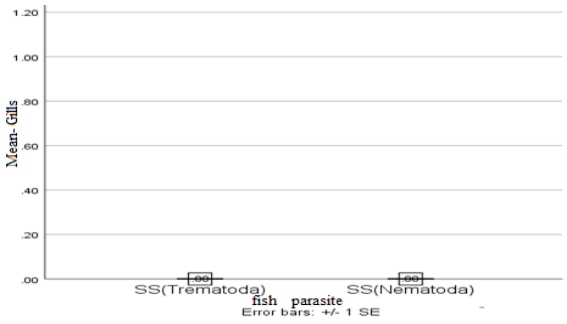

Figure (4) shows the average infection of the parasites Trematoda and Acanthocephalan in the gills of (SS): *S. salpa*.

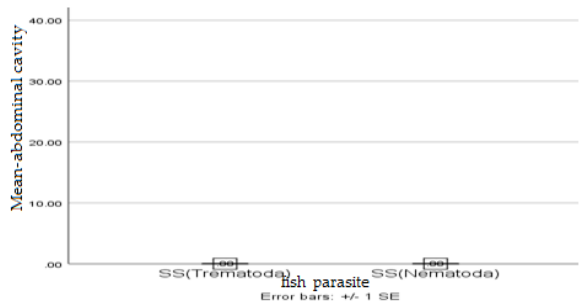

Figure (5): shows the average infection of parasites Trematoda and Acanthocephalan in the abdominal cavity of (SS): *S. salpa*.

Figure (6) indicates the average infection of Acanthocephalan and Trematoda parasites in the stomach of *S. salpa* fish, where the average infection of Trematoda was recorded as (2.00±0.25), while there were no recorded infections of acanthocephalan. Figure (7) examination of the anterior intestines of *S. salpa* fish showed that the average infection with Trematoda parasites was 2.70±0.30, while the infection with Acanthocephalan was absent. While, examination of the posterior intestines of *S. salpa* fish showed that the average infection of Trematoda parasites was 4.00 ± 0.47, while the infection of Acanthocephalan was recorded at an average of 1.20 ± 0.13 (Figure 8)

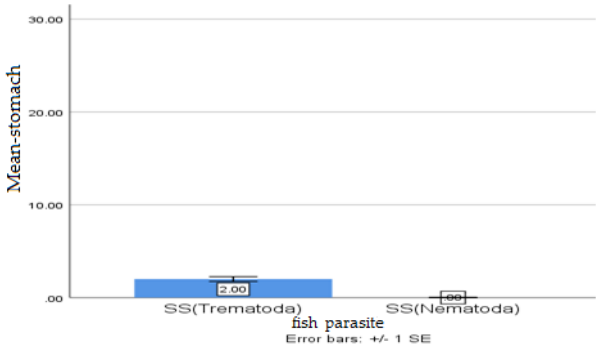

Figure (6): Average infection with the parasites Acanthocephalan and Trematoda in the stomach of *S. salpa*.

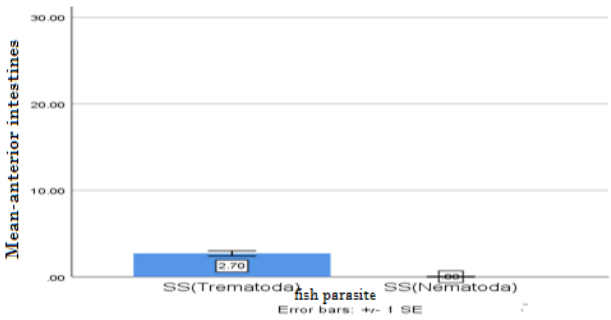

Figure (7): Average infestation of parasites Acanthocephalan and Trematoda in the anterior intestines of (SS): *S. salpa*.

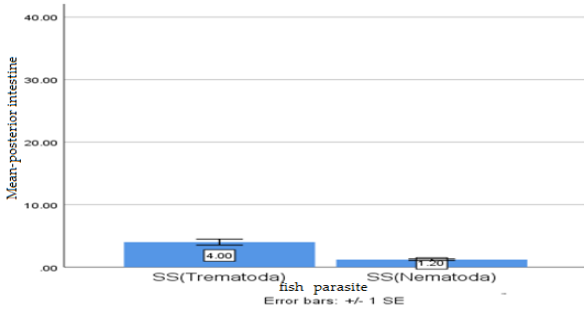

Figure (8): Average infection with parasites Acanthocephalan, and Trematoda in the posterior intestine of *S. salpa*.

By examining the liver of *S. salpa* fish, Figure (9) showed that the average infection with Trematoda parasites in the liver was 0.16±1.60, while the infection with acanthocephalan parasites was absent. Through microscopic examination of the spleen (Figure 10), it was found that there was no infection with any type of helminths parasites, whether Acanthocephalan or Trematoda, in *S. salpa* fish

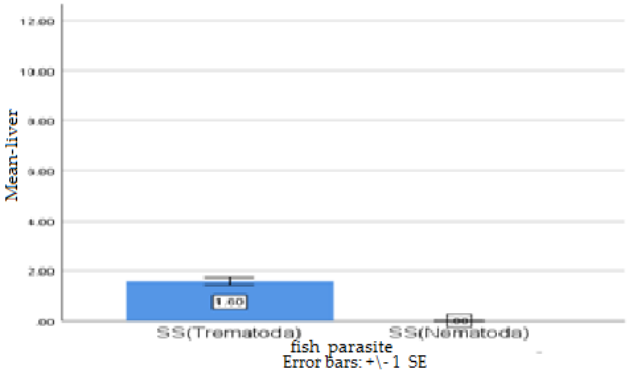

Figure (9): Average infection with parasites Acanthocephalan, Trematoda in the liver of *S. salpa.*

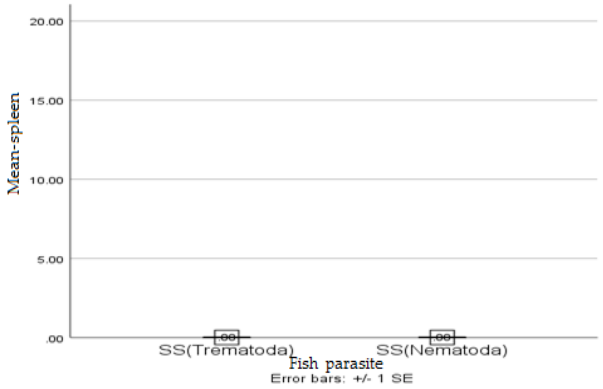

Figure (10): Average infection with parasites Acanthocephala, and Trematoda in the spleen of *S. salpa*.

Histological examination of samples of *S. salpa* fish showed parasitic infections with Trematoda worms, which led to damage and tearing of the secondary gill filaments (Figure 11, A). The same form indicates disintegration in the intestinal tissue and an increase in the size of some epithelial cell nuclei (Figure 11, B). Histological examination of liver samples showed significant tissue disintegration and clear fibrosis (Figure 11, C).

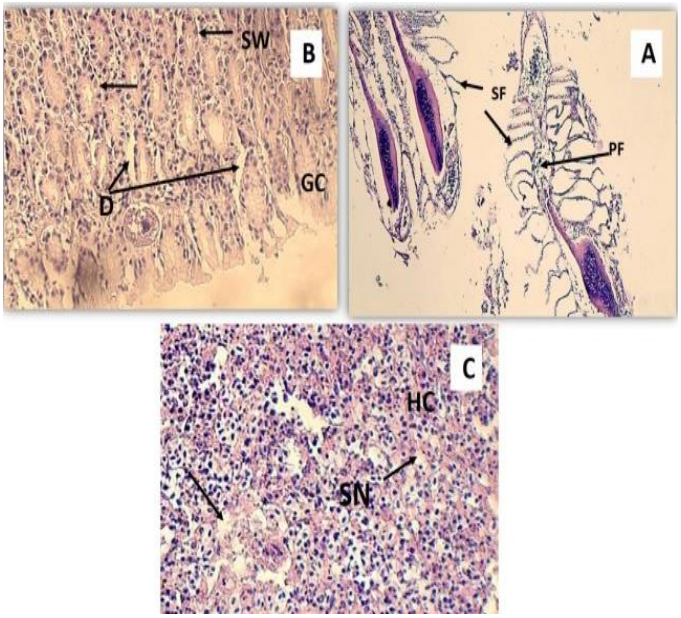

Figure (11): Parasitic infection of *S. salpa* (H&E): A-Gills,(PF): Primary gill filaments ,(SF): Secondary gill filaments:(↑), Laceration.(40X).B-Intestines,(GC):Goblet cells),SW):Cellular swelling(↑): Nuclei of epithelial cells. (400X). C- Liver), HC): Hepatic cells), SN): Venous sinusoids, (↑):Tissue decomposition. (200X).

4 Discussion

The results of this study showed the presence of various infections with helminths in the studied fish samples, where the overall infection rate for *S. salpa* was 100%. These results were consistent with what was found by Alabbar (2014), where the overall infection rate for parasites was 95.0% and 100% in species of bony fish (Herlyn *et al.*, 2003). Meanwhile, the monthly infection rate in the current study fish reached 30%, and this result is similar to the study by Shamsi (2018), where the monthly infection rate was 34.3%.

The results showed that during the external examination of *S. salpa*, no presence of any external helminths. Meanwhile, the external infection rate of helminths was absent, and there were no external changes in the appearance of the fish. These results agreed with the results of the study done by Mohammed *et al.*, (2017), during the external examination of the collected *Microine epinephelus*, no external parasites were found, unlike the results of the study done by Hussein (2017), where different external parasites were present in 122 fish samples with minor infections on the skin, gill covers, and fins. The reason for these discrepancies could be attributed to the differences in fish species, sample sizes, and study seasons. Additionally, the microscopic examination of the study's fish gills showed an absence of parasitic infections (0%), which contradicts the findings of Yousry *et al.*, (2019), where *Euthynnus alletteratus* fish had an infection rate of 0.67± 0.41 with Acanthocephalan and Trematoda parasites. Furthermore, the current study did not match the findings of Abdul-Ameer (2010), who recorded two types of Trematoda in the gills of *A. vorax* and *B. sharpeyi* fish.

The statistical analysis of the data indicated that the incidence of Trematoda and Acanthocephalan in the abdominal cavity of the studied fish was (0%). These results were consistent with the study conducted by Salman *et al.*, (2010) on Mugilidae fish to determine some species of Trematoda, where no helminth infection was recorded upon examination of the abdominal cavity. In contrast to what Yousry *et al.*, (2019) indicated, where the infection rate of the Acanthocephalan parasites was 32.2±1.52, while no infection of the Trematoda parasite was recorded in *E.alletteratus* fish. Hui-Shih (2004) also recorded helminth infections in the abdominal cavity in his study on fish *Trichiurus lepturus*. Similarly, Al-Taee, and Zangana (2011) in their study on marine fish species (Cyprinidae) recorded two types of Nematoda in the abdominal cavity, as Alabbar (2014) indicated a high incidence of helminths in the abdominal cavity of *M. helena* fish, at a rate of (47.28%). Many studies suggest that the density of infection is associated with seasonal changes, and therefore the density of infection may be higher in the summer and lower during the winter season based on the activity of the parasite (Kundu, *et al* 2015; Sures *et al.*, 2003; Hui-Shih, 2004).

figure (6) indicates the average infection of Acanthocephalan and Trematoda parasites in the stomach of *S. salpa* fish, where the average infection rate of Trematoda was recorded (2.00±0.25), while no infection by Acanthocephalan was recorded. This is consistent with the study conducted by Parveen *et al.*, (2018) on a type of marine fish, *P. scinctus*, which was recorded as a Trematoda parasite infection in the stomach, leading to tissue damage. In contrast, Yousry *et al.*, (2019) reported in their study on *E. alletteratus* fish and their infection with Acanthocephala and

Trematoda parasites in the stomach, where the average infection rate of the first type was 25.40±1.52, while no infection by the second type of parasites was recorded.

It was observed that the infection rate of intestinal parasites in the anterior and posterior intestines of the fish in the current study was 30-31% respectively, these results were close to the study conducted by Al-Alusi, (2011), which included the intestines of (carp) fish, with an infection rate of 29.8% and 34.6%, respectively. The results were also somewhat consistent with those of Al-Taee, and Zangana (2011), who recorded a parasitic infection of Nematode in the intestines of *ALburnus capito* with a rate of 20%. In a study conducted by Kennedy (1976), the infection rate in the anterior part of the intestines of salmon was 14%, while the infection rate in the posterior part varied and reached 46%. Alabbar (2014) noted in his study on internal helminths that infect a type of marine fish that the infection rate in the anterior intestines was 13.95%, while it was 9.86% in the posterior intestines.

In examining the liver of *S. salpa* fish, the results showed that the percentage of parasitic infection in the liver was 17%, which corresponds to the study conducted by Alabbar (2014) that indicated the presence of parasitic infection in the liver of a type of marine fish at a rate of 16.33%. The results of this study also showed that the average infection rate with the Trematoda was 0.16±1.60, while the infection with the Acanthocephala parasite was absent. This is contrary to what was reached by Al-Taee, and Zangana (2011) who recorded an infection with Nematode in the liver of *A. capito* fish at a rate of 20%.

Through microscopic examination of the spleen, it was found that there was no infection with any type of helminths, including Acanthocephala and Trematoda, in *S. salpa* and that is consistent with the study conducted by Alabbar (2014). No cases of internal helminths were recorded in the spleen of *M. Helena* fish. However, this study did not agree with the study conducted by Blazer *et al.,* (2010), where helminth infections were recorded in the spleen of (sunfish) at moderate to high rates.

The study conducted by Salman *et al.* (2010) on the Burian family fish (Mugilidae) to identify Trematoda infection, the study presented that the seasonal and monthly variations affect Trematoda's distribution and spread. They appeared in all seasons of the year, but with different rates, with the highest infection rate recorded during the summer and spring seasons, While the lowest rate of infection with these worms was recorded during the winter, indicating the clear effect of seasonal changes in temperature on the rate and severity of Trematoda infection. On the other hand, parasites are essential components in animal communities, and they are usually more abundant than their hosts. In addition, completing the life cycle of many parasitic species requires various models of vertebrate and invertebrate animals that act as intermediate or final hosts. Mollusks are a common intermediate host for these parasites. Therefore, the change in the composition of parasitic communities reflects the change in the composition of marine animal species (Galli *et al*., 2001). In general, the differences that occur in the rate and percentage of infection are due to many reasons. The reason for the difference may be due to the nature of the nutrition of the host, as well as differences in feeding habits and not being in the same environmental conditions (Kennedy,1976), or the reason for the presence of differences in infection is due to the size of the sample and its inclusion for different lengths or studied over different months، Since both the proportion and severity of the injury increases with the length and size of the fish or with the age of the fish (Taher *et al.,* 2017).

The histological examination of samples of *S. salpa* fish showed the presence of helminths, which led to damage and tearing of the secondary gill filaments (Figure 11, A). The same figure also indicates the breakdown of intestinal tissue and an increase in the size of some epithelial cell nuclei (Figure 11, B). The histological examination of liver samples from the fish studied showed significant breakdown of the liver's basic tissue and clear fibrosis of its tissues (Figure 11, C). These results are consistent with the study of Sures *et al.,* (2003), which indicated tissue breakdown in (salmon) due to parasitic secretions and the fish's immune resistance to infection. helminths, when found in fish, migrate extensively, causing tissue changes in various organs, leading to direct tissue damage and the onset of hypersensitivity reactions. Feeding of threadworms on host tissues is considered an important cause of various diseases (Parveen *et al*., 2018). The current study agrees with the study conducted by Parveen *et al.,* (2018) which revealed from a histological examination of the stomach of *P. cinctus* fish that it was infected with a number of helminths and a contagious tumor in the mucous membrane with complete destruction and damage to the mucous epithelial. In addition to these changes, the entire structure of the villi was completely destroyed and shrunk, causing a change in the morphology of the stomach. Pardeshi *et al.,* 2012 indicated in their study of tissue injuries in a type of marine fish liver caused by the Trematoda parasite that the anatomical examination of the affected liver revealed the presence of abscesses associated with the serous membrane of the affected liver and the presence of a large cyst filled with eggs that caused disruptions in

the vital functions of the glands. These disruptions may directly affect the chemical nature of the affected tissues, thus damaging the liver tissues and changing their shape and size.

5 Conclusions

From this study, it can be concluded that there are infections of some helminths in *S. salpa* fish, and the rate of prevalence of Trematoda was higher compared to acanthocephalan. The highest infection rate was recorded in the posterior and anterior intestines, followed by the stomach, and then the liver. As for the spleen, gills, abdominal cavity, and ectoparasites, the infection rate was absent. Examining the tissue sections revealed the presence of various infections in fish organs (gills, liver, and intestines).

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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The Effect of Poultry Manure on Growth, and Yield of Tomatoes (*Lycopersicon esculentum* mill) Cultivated in Salt Marsh Soil

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A B S T R A C T

Tomato is an important, popular, and versatile vegetable in the world and ranks number one in its contribution to the diet. One of the most common land degradation processes that affect agricultural production is soil salinization, however, organic production can be utilized to reduce the effect of salinity on many plants. The study aims to investigate the effect of different concentrations (0, 25, 31, 38, and 44%) of poultry manure (PM) on cherry tomato plants grown in marsh soil and to study the effectiveness of fertilizer in improving soil properties. The results showed that the application of PM in marsh soil increased plant height, root length, fresh and dry weight, the number of flowers and fruits and shoot potassium concentration, while shoot sodium concentration was decreased. The present study concluded that treating salt marsh soil with PM levels especially with PM4 level could reduce salinity stress damage on cherry tomato plants, increase biomass production and improve soil properties.

1 Introduction

One of the basic plants in the diet of most people in the world is the tomato plant, Lycopersicon esculentum, (Ohlson *et al*., 2018) which belongs to the Solanaceae family (Ohlson *et al*., 2018). Tomato is enriched with minerals, vitamins, sugars, dietary fibers, essential amino acids, and lycopene (Ud Din *et al*., 2023). It also contain high levels of other bioactive compounds such as phenolics, vitamin C, and antioxidants which are thought to protect and possibly prevent stressful environmental conditions (Najat *et al*., 2018). Lycopene is a natural antioxidant that works effectively to slow the growth of cancerous cells (Ntagkas *et al*., 2020). Tomatoes help maintain strong bones. This is because they contain considerable amounts of calcium and Vitamin K. Both nutrients are essential in strengthening and performing minor repairs on the bones as well as the bone tissue (Amao, 2018). The total area of the world under tomato cultivation is 4.8 million hectares (MHA) with the production of 163 million tons per year (Firdous, 2021). Destructive climate changes are resulting in the degradation of soil, which eventually lowers the overall crop productivity.

Globally, soil salinization is one of the most important issues that affects almost 836 Mha (Xie *et al*., 2020). Salt-affected soils reduce crop yields by disturbing the physicochemical properties and microbial activities in the soils of semi-arid regions (Zhu *et al*., 2021). Salinity

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is one of the most important abiotic stresses that reduce plant growth and development due to the presence of salts in the soil (Zörb *et al*., 2019). Plant roots absorb Na+ and C1− ions from the soil solution and translocate them in the stem and leaves. Due to specific ion toxicity, dehydration occurs which reduces the photosynthetic and respiration activities in plant cells (Acosta-Motos *et al*., 2017). Furthermore, When salts increase in the soil sector, the osmotic pressure in the area of root spread increases, and for the plant to be able to resist these unfavorable conditions in the soil solution, the plant cells raise the internal osmotic pressure of the cytoplasm, and this leads to the plant loses the vital energy necessary for its development and growth (Kim *et al*., 2016), which leads to its weakness and lack of production (Ivushkin *et al*., 2019; Luo *et al*., 2005) Nowadays, different organic amendments such as compost, farmyard manure, biochar, animal manure, and crop residues are being used to mitigate the drastic effects of salinity.

Organic materials such as poultry manure enhance crop yield and soil properties and sequester more carbon (C) in soils(Ud Din *et al*., 2023). Also, Organic materials reduce the salinity stress, Na+ adsorption ratio, and electrical conductivity (EC) by improving the physical, chemical, and biological characteristics of highly deteriorated soil for sustainable crop production (Guo *et al*., 2020). The addition of compost to soil influences plant growth positively even in a stressed environment. Research conducted by (Soremi *et al*., 2017) showed that PM is the most cherished of all animal manures since it contains all the essential plant nutrients such as phosphorous, nitrogen, potassium, zinc, iron, chlorine, calcium, magnesium, boron, copper, molybdenum and sulfur which are responsible for the fertilization of the soils. This makes it the most suitable organic manure for tomato production.

Under salt stress, plants are unable to uptake balanced amounts of nutrients and water from the soil, but the addition of compost improves the organic matter of the soil and provides more surface area for microbes to chelate the salted ions (Cao *et al*., 2019). Moreover, the presence of more functional groups and active sites in compost attracts the salted ions and makes them unavailable for plant intake (Gondek *et al*., 2020). The response of plants to environments with high salt content is one of the most important agricultural topics that botanists are interested due to its close connection to the source of human food. In addition, our country Libya suffers from a high rate of salinity in its lands, we have chosen to conduct this study on one of the important crops of economic and medical importance, which is the tomato plant, to evaluate the effect of poultry manure on the growth and production of cherry tomatoes (Lycopersicon esculentum mill) in salt marsh soil under environmental conditions.

2 Materials and Methods

2.1 Experimental Site

The study was conducted at the Faculty of Science in Botany's department, Misurata, Libya to determine the effects and interactions of marsh soil and PM applications on tomato growth.

2.2 Seed Source

The plant identification was carried out by the author following these references; Cronquist, 1968; Group, 2009). The plant was identified in the plant herbarium of the Botany Department, Faculty of Science, Misurata University. The name of the plant used was *Lycopersicon esculentum* mill.

2.3 Soils Sample Collection

The soil samples of the experiment were collected from marsh soil of the Qasr Ahmed area which is located between longitude (32°15`&32°23`N) and latitude (15°10`&15°16`N) in Misurata/Libya (Assoul *et al*.).

2.4 Levels used in the Study

The control: saline soil without adding PM (0%), and it is symbolized by MP0.

The first level (25%): 1 kg of PM was added to 3 kg of soil, and its symbol is MP1.

The second level (31%): 1.25 kg of PM was added to 2.75 kg of soil, and it is symbolized by MP2.

The third level (38%): 1.5 PM were added to 2.5 kg of soil, and it is symbolized by MP3.

The fourth level (44%): 1.75 kg of PM was added to 2.25 kg of soil, and its symbol is MP4.

2.5 The Edaphic Parameters

The soil analyzed before and after cultivation to evaluate their degree of fertility and granulometric compositions. From the soil, four samples were taken at a depth of 30 cm (about 11.81 in) and then dried

(Alghobar & Suresha, 2017; Disciglio *et al*., 2015), crushed with a mortar, sieved to 2 mm (about 0.08 in), and finally subjected to chemical analysis.

2.6 Soil samples physicochemical analysis

2.6.1 Determination of the pH Values and Electrical Conductivity (EC) of the Soil

The soil electrical conductivity (mmhos cm-1) and PH were measured on aqueous soil extracts (ASE) using an electrical conductivity meter and pH meter. The accuracy of the device has been verified using a 0.01 N KCL solution, which gives a conductivity reading of 1.413 ds/m at a temperature of 25 °C (Page *et al*., 1982) and electrical conductivity (EC) was measured in a 1:10 soil/water solution using an EC meter (Jenway, United Kingdom) (Rayment & Higginson, 1992). The size of the soil particles was determined using a 2 mm sieve, through which the soil texture can be known (Gee & Or, 2002)). The analysis was conducted at the Misurata Agricultural Research Center, as shown in (Tables 1 & 2).

Table (1): Chemical properties of the Qasr Ahmed marsh soil before cultivation

Texture of the Soil	PH	EC ms/cm	Sodium (Na) (ppm)	Potassium (K) (ppm)
Mixed (sandy clay)	9.9	0.448	8232.8	1321.0

Table (2): Chemical properties of the Qasr Ahmed marsh soil after cultivation.

Levels of PM	PH	EC ms/cm	Sodium (Na)/(ppm)	Potassium (K) /(ppm)
PM0(control)	9.9	0.493	85.46	5.95
PM1	7.9	1.207	62.67	9.04
PM2	7.7	1.008	63.05	9.04
PM3	7.2	1.056	55.07	9.9
PM4	7.4	0.637	49.37	9.78

2.7 Experimental Details

The vitality of the seeds of the cherry tomato plant (*Lycopersicon esculentum* mill) was tested, and their germination rate reached 99%. The tomato plant was grown in the greenhouse, and after 6 weeks (about 1 and a half months) of cultivation, when the fourth leaf appeared, the seedlings were transferred in single form to pots with a diameter 20 cm filled with soil mixed with the PM to be studied for its effectiveness. The samples were irrigated with fresh water 2 to 3 times a week. During the follow-up period of tomato plant growth, some morphological and chemical parameters were studied.

2.8 The Morphological Characteristics

2.8.1 Plant Height (cm)

Plant height was taken as the length between the bases of the plant to the tip. Plant height was recorded using a graduated ruler (cm) every two weeks until the end of the growing season.

2.8.2 Number of Leaves and Branches Per Plant

This parameter was measured by calculating all leaves of ten randomly selected plants at different stages of growth at all levels studied. The mean of leaves per plant was recorded.

2.8.3 Number of Flowers and fruits Per Plant

The number of flowers and fruits formed at all levels of the studied PM, from its formation to the end of the experiment, was counted.

2.9 Fresh and Dry Weight of Plant (g):

Plants were harvested after twenty weeks of treatment and shoots were washed with distilled water. Patted dry with paper towels and quickly weighed for determination of fresh weight. Dry mass was determined after drying in an oven (Ohaus) at 80 ^{o}C for 3 days then weighed using a sensitive Metler AND (HR-60) balance.

2.10 Extraction of Plants and ion Analysis

To determine different mineral elements (K and Na), tomato sample collected from each pot was cut into small pieces using a sharp steel knife and dried in an electric oven at 50°C temperature for about 72 hrs. Then the samples were ground by a grinding mill and used to prepare tomato sample extract by wet oxidation method using a di-acid mixture. Na and K were estimated by flame photometrically at wavelengths 589 and 767 nm, respectively as mentioned by Singh *et al*. (1999).

Statistical Analysis

Data were analyzed by two-way analysis of variance (ANOVA) to explore the general trend of the experimental data. SPSS (version 20) statistical software package (SPSS, Chicago, USA) was employed in the

analysis. The means were separated using the Tukey method of the multiple range test at a 5% level of probability.

3 Results

3.1 Plant height, Leaf Number and Branch Number

In the present study, the application of PM in marsh soil had a significant effect (P≤0.05) on the growth parameters of *L. esculentum* mill plants as compared to the control during all weeks of the study (Table 1). Plant height, leaf number and branch number increased in response to PM treatments. Plant height and leaf number were increased with increasing PM levels in the growth media. It result in a (50%) increase in shoot height and leaf numbers at PM4 as compared to the control. While the branch number was maximal at PM2, it was 65 % higher than that at control.

Table (3): Plant height, leaf number and branch number in *L. esculentum* mill grown under different levels of PM (0, 25, 31, 38 and 44%), in salt marsh soil.

Duration (week)	Treatment (PM %)	Plant height	Leaf number	Branch number
8	PM0 (0%)	18 ± 0,4c	4.4 ± 0.7d	0.6±0.03c
	PM1 (25 %)	32 ± 0.2b	7.6 ± 0.6b	1.6 ±0.2b
	PM2 (31%)	33 ± 0.2b	9.6 ±0.5b	2.8 ±0.4a
	PM3 (38%)	38 ± 0.2a	9.6 ± 0.6c	1.6 ±0.2b
	PM4 (44%)	39 ± 0.5a	11.8 ± 0.5a	1.6 ±0.02b
12	PM0 (0%)	34 ± 0.4d	7.8 ± 0.1a	1.4 ±0.1d
	PM1 (25 %)	43 ± 0.5bc	10.4 ±0.5b	3.2 ±0.3b
	PM2 (31%)	44 ± 0.4c	11.4 ±0.6b	4 ±0.3a
	PM3 (38%)	47 ± 0.5b	11.2 ± 0.5b	2.6 ±0.2c
	PM4 (44%)	62 ±0.6a	14.6 ± 0.4a	3.4 ±0.1b
20	PM0 (0%)	41 ± 1d	9.4 ± 1d	1.4 ±0.1c
	PM1 (25 %)	54 ± 0.5c	13.4 ± 0.6c	3.2 ±0.3b
	PM2 (31%)	56 ± 0.3b	15,4 ± 0.5c	4 ±0.2a
	PM3 (38%)	58 ± 0.7b	17± 0.3b	3.4 ±0.1b
	PM4 (44%)	82 ± 0.8a	18.8 ± 0.2 a	3.4 ±0.2b

Plants were grown for 8, 12 and 20 weeks under environmental conditions. Values are means ± standard error (n = 5). Letters indicate a significant difference in means from post hoc Tukey tests (P < 0.05).

3.2 Root Length (cm)

Fig. 1 showed that PM application in salt marsh soil had a significant effect (P≤0.001) on the length of root of *L. esculentum* mill plants. The root length increased with increasing PM levels in the growth media, with the highest value recorded at the PM4 level, which was 1.6 times higher than the plant growth in the control.

3.3 Number of Flowers and Fruits

The application of PM in salt marsh soil had a positive impact on flower and fruit production in *L. esculentum* mill plants, as demonstrated in Fig. 2A and b. The number of flowers and fruits plant per plant was maximally enhanced at the PM4 level, showing an increase of 69% and 80% in flower and fruit yield, respectively, as compared to the control plants.

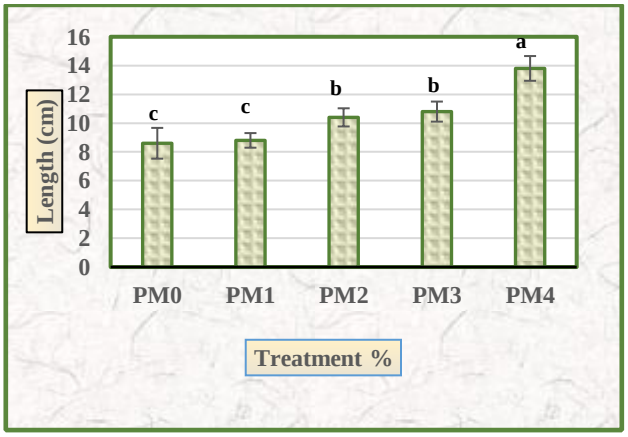

Figure (1): Root length of *L. esculentum* mill plants grown under different concentrations of PM (0, 25, 31, 38 and 44 %), in salt marsh soil, for 20 weeks in environmental conditions. Letters above error bars (n = 5) indicate a significant difference in means from post hoc Tukey tests (P < 0.05).

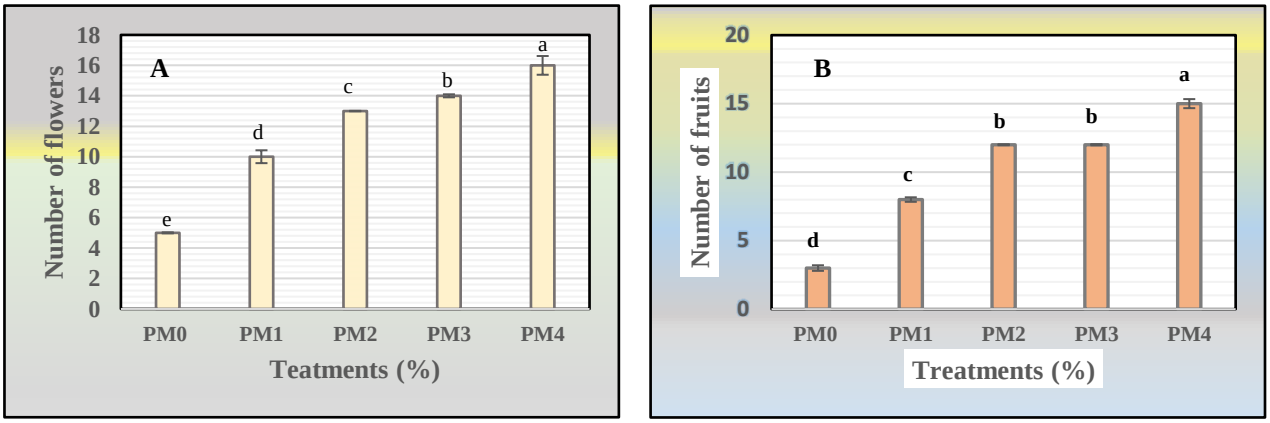

Figure (2): Flowers (A) and fruits (B) numbers of *L. esculentum* mill plants grown under different concentrations of PM (0, 25, 31, 38 and 44 %), in salt marsh soil, for 20 weeks in environmental conditions. Letters above error bars (n = 5) indicate a significant difference in means from post hoc Tukey tests (P < 0.05)

3.4 Plant fresh and Dry Weight

Fig. 3. Showed the effect of different levels of PM on fresh and dry weight for plants grown in marsh salt soil. The results showed that the effect of PM levels was highly significant (*P*< 0.01) on the fresh and dry weight plant per. The fresh weight increased with the increasing PM level in the growth medium. The highest value (23±1 g per plant) was at PM4, and the lowest value (5±0.02g per plant) was at PM0 (control treatment). Furthermore, a similar pattern of response to the application of PM in growth media significantly affected shoot dry weight (p < 0.01; fig. 3), with the highest value (8±0.2g per plant) was recorded in PM2 than in PM0 (1.7±0.02g per plant)

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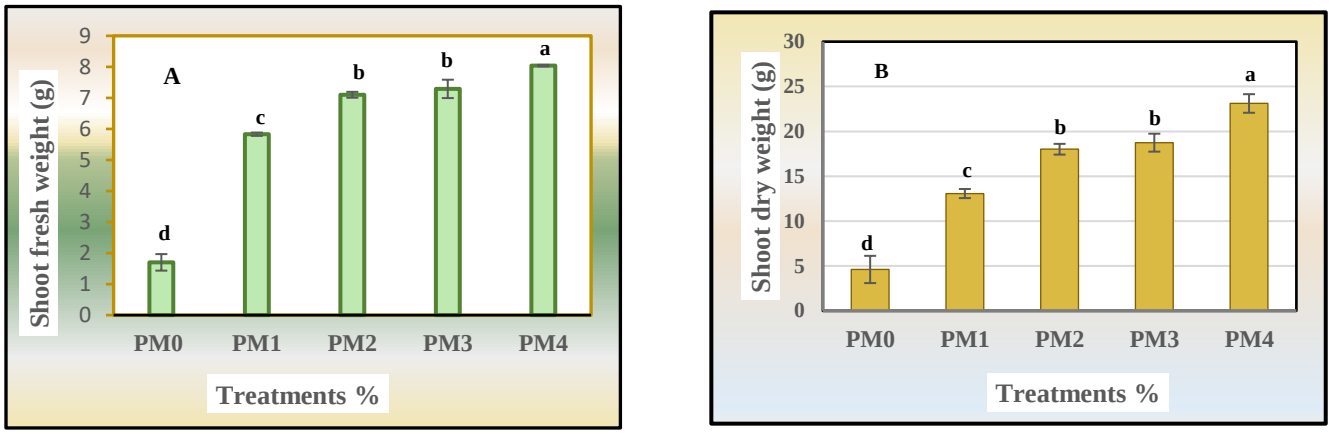

Figure (3): Shoot fresh (A) and dry weight (B) (g per plant) of *L. esculentum* mill plants grown under different concentrations of PM (0, 25, 31, 38 and 44 %), in salt marsh soil, for 20 weeks in environmental conditions. Letters above error bars (n = 5) indicate a significant difference in means from post hoc Tukey tests (P < 0.05)

3.4.1 Ions Concentrations

Shoot K^+ and Na^+ concentrations were significantly affected (*P < 0.001*) by the application of PM in salt marsh soil. As shown in Table 4; the shoot K^+ concentration was increased by increasing PM levels, at the PM4 level; the K^+ concentration was 1.4 times greater than at the control (PM0). However, the shoot Na^+ concentration tended to be reduced significantly (*P < 0.001*) by an increased PM level in the growth medium, which was 2.2 times lower as compared with the control.

Table (4): Concentration of sodium and potassium in tomato plants treated with different levels of PM.

Treatments (%)		Sodium (Na^+) (ppm/g^{-1})	Potassium (K^+) (ppm/g^{-1})
PM0	(0%)	49.22±0.03a	40.41±0.01 e
PM1	(25 %)	37.05±0.02b	48.06 ±0.03d
PM2	(31%)	33.96 ±0.02c	50.41±0.12 c
PM3	(38%)	29.46±0.14d	56.60±0.03 ab
PM4	(44%)	22.71±0.03e	58.01±0.04a

Significantly, different means are labeled with different letters at $p < 0.05$ (ANOVA followed by Tukey's test).

4 Discussion

Results from this study showed a decrease in the morphological characteristics and yield parameters of tomato under salinity conditions. However, the application of PM reduced the negative impacts of salinity and improved plant growth. In comparison with the control, poultry manure-treated plants showed an increase in plant height (Table 3). The treated plant with PM resulted in higher growth, suggesting that fertilization enhanced the growth of tomatoes. This agreed with the work of Direkvandi *et al*., (2008) and Ayeni *et al*., (2010), who reported a significant increase in plant height as a result of the application of PM. In addition, the application of PM during the growth of the plant under salt-stress increased the number of leaves. A similar result was found in the findings of Singh *et al.*, (2020) and Agbede *et al*., (2008), who found that the application of PM led to an increase in the number of tomato leaves. Our results were also consistent with the research outcomes of Kumar *et al*, (2021) who reported that the reduced number of branches of the *Oenanthe javanica* plant was due to an increased concentration of salts in the growing medium.The increase in the number of branches is consistent with Ewulo *et al*., (2008) study of the effect of PM at concentrations of 0, 10, 25, 50, and 40 tons/ha on tomato growth. The possible reason behind the stunted growth is due to the decreased absorption of essential nutrients in the plant (Zhang *et al*., 2019). High salinity in plants alters cell division, decreases cell size by decreasing water potential, and eventually reduces the dry weight of plants (Safdar,*et al*.,2019). The number of flowers and fruits in tomato plants indicated that there were significant differences ($p<0.05$) among the different rates of PM throughout the growing period. This result is in line with the findings of Agbede *et al*. (2008), who found that the number of fruits in crops significantly increased with an increase in the concentration of PM.

Reduced weight might be due to various other factors, including decreased photosynthetic activity and a drop in turgor pressure under salt stress (Ors *et al.*, 2021). On the other hand, the addition of PM in marsh soil minimized the toxicity of salts to plants, either directly by decreasing the translocation of harmful salts or indirectly by increasing other nutrients (Castiglione *et al.*, 2021). The result here indicates that all levels of PM improve fresh and dry weight compared to the control treatment. These results were supported by Abu Suwar and El Zilal (2010), who stated that fresh and dry weights were increased significantly by PM. The highest dose of manure was used to achieve the maximum dry weight. These results also agreed with Kandil and Gad (2010). In addition, a similar result was found with Aziz *et al*., 2020.

The results of the chemical parameters of the soil samples presented in Tables 1 and 2. The obtained pH values indicated that soil samples before treatment with PM levels were strongly alkaline (Ph 9.9). However, after treatment with PM levels, the pH values reduced with the increase in PM levels. The incorporation of PM lowered the pH of saline-sodic soil due to its acidifying effect by producing and subsequently releasing different organic acids during the mineralization process of the nutrients. The pH of alkaline soil is controlled by Na. However, when PM is used as a source of nutrients, it made free Ca^{2+}, which led to a reduction in soil pH. As a result, Na^+ ions released in soil solutions that were leached down the soil profile (Brady & Weil, 2005). This may be attributed to the sufficient release of nutrients particularly N.P.K contained in the PM applied, as these nutrients improve the growth and yield of crops under stressful environmental conditions.

Potassium plays an important role in different mechanisms like protein synthesis, glycolytic enzymes, and photosynthesis. Treatment plants grown in marsh soil with PM improved the growth rate and productivity of tomato plants growth. This may be due to an increase in the plant's potassium concentration. The increase in the K^+ concentration of tomato due to the application of poultry manure is consistent with using PM as fertilizer for tomato production (Akanni, 2005). It was found that 30 t ha^{-1} PM gave the most growth and highest fruit yield among all PM levels. The reduction in K^+ and the increase in Na^+ shoot concentrations after salinity treatment is because that K^+ and Na^+ are inherently

competitive due to their similar physiological properties. Na^+ competes with K+ for binding sites in the cytoplasm, inhibiting K^+-dependent metabolic processes.

5 Conclusions

It is clear that, using different levels of poultry manure is useful for enhancing the growth, development, and yield of tomato crop production under salty soil. In addition, the application of PM in marsh soil has led to improvements in the physical characteristics of the soil and minimized the toxicity of salts to plants, either directly by decreasing the translocation of harmful salts or indirectly by increasing other nutrients. Thus, the best growth was at the highest PM4 level in the growing medium. The study recommend using saline soil and treating it with PM to grow tomatoes (cherry tomatoes). Further research and further investigation are needed on other plants using different concentrations of poultry manure available to improve the specifications and properties of marsh soil.

Contrast of Interest: The authors declare that they have/ have no contrast of interest associated with this manuscript.

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A survey study of the characteristic, causes, and symptoms of anemia among pregnant women and its effects on women and fetus

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A B S T R A C T

Pregnancy is a physiological condition characterized by a high demand for energy and an increased need for oxygen. This demand escalates as pregnancy progresses to meet the requirements of both the mother and the fetus. Anemia, on the other hand, results from an imbalance in red blood cells is often cause by iron deficiency. When iron levels are low, it leads to a decrease in hemoglobin, which, in turn, impairs the body's ability to provide the necessary oxygen for its vital processes. The study involved 96 pregnant women who surveyed using a questionnaire. Among them, 74 exhibited signs and symptoms of anemia, while 22 were included in the control group. Additionally, through the calculation of proportions, we analyzed the symptoms that later manifested in both the mothers and the fetuses. It discovered that the anemia was most prevalent in the age group of 36 to 45. The results of the research sample indicated that miscarriages and stillbirths occurred, although in relatively low numbers. The most prominent symptoms reported by the pregnant women with anemia were headaches and dizziness, while the affected fetuses exhibited jaundice and low blood pressure. In brief**,** anemia is a condition that can affect pregnant women of all ages and often caused by a lack of access to health information and an inadequate diet for their stage of pregnancy.

1 Introduction

Numerous normal physiological, hormonal, metabolic, and psychological changes take place throughout pregnancy. While there is not a one-size-fits-all diet for pregnant women, they do require additional energy and nutrients due to the growth of the baby, placenta, and other supporting tissues during this period maternal malnutrition during pregnancy can lead to several adverse outcomes, including premature birth, low birth weight, and congenital abnormalities in the fetus. These complications can pose a risk to the infant's survival (Al-Dulaimi, 2016). Anemia is a global public health concern that an impact both developing and industrialized nations, with significant repercussions for human health. It also represents a social and economic burden. Anemia characterized by a reduction in the quantity or quality of red blood cells (RBCs). This reduction in the capacity of red blood cells to transport an adequate amount of oxygen can lead to insufficient oxygen supply for the diverse biological systems, which vary depending on factors such as age, gender, and pregnancy. Historically, anemia was primarily, attributed to iron deficiency, believed to be the most common cause globally. However, anemia can also be the result of insufficient folic acid, vitamin B12, or vitamin A, as well as recurrent infections (Bekele *et al*., 2016), and it may be linked to parasite infections and genetic disorders. According to

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the WHO, anemia characterized by a reduction in the number and size of red blood cells or a decrease in hemoglobin levels below the normal range, leading to a diminished capacity of the blood to transport oxygen throughout the body (WHO, 2014). The severity of the condition also influenced by the symptoms it presents. Common symptoms of anemia include headaches, lightheadedness, drowsiness, pallor, palpitations, shortness of breath, and difficulty in focusing (Fishbane and Spinowitz ,2018) Acute anemia occurs when there is a rapid reduction in red blood cells, often due to acute bleeding or hemolysis. On the other hand, chronic anemia develops gradually due to a consistent decline in the number of red blood cells. This decline can be attribute to factors such as iron deficiency, nutritional deficiencies, or chronic medical conditions. (Killeen and Tambe, 2023). Anemia affects people of all ages, but pregnant women are the most vulnerable (Mettananda *et al.*, 2018Anemia is a widespread global concern, affecting approximately 2.1 billion people, including 6 million pregnant women. Alarmingly, it stands as the leading cause of maternal fatalities in roughly 20% of cases (Jiji and Rajagopal, 2014). Furthermore, anemia impacts nearly one-third of the world's population, with over half a billion women of reproductive age affected globally (WHO, 2004). The regions of South Asia, Central Africa, and West Africa exhibit some of the highest incidence rates. In 2011, among the 32.4 million pregnant women aged 15 to 49 worldwide, approximately 496 million (29%) were anemic (Stevens *et al*., 2013).

Objective: The current study was to investigate some of the characteristics and symptoms and effective of anemia encountered by pregnant women and their fetuses.

2 Materials and Methods

In the current study focusing on pregnant women, we employed a questionnaire to collect data on pregnant women with anemia and assess the impact of anemia on their pregnancies, fetuses, and childbirth outcomes. The study included 96 participants, with 74 of them exhibiting signs and symptoms of anemia, while the remaining 22 served as the control group (comprising healthy pregnant women). The questionnaire was design to encompass the following sections: age of anemia onset, underlying causes, maternal pathological symptoms, association with miscarriages, impact on newborns, and potential fetal outcomes including deformities.

3 Results

1- **The age** at which the injury occurred: According to the findings of the present study, ages 17 to 25 had the lowest risk of disease, while ages 36 to 45 had the greatest prevalence. (Table: 1), (Figure: 1).

Table: **(1**) Shows the percent % of ages at which the anemia occurred.

Age	Frequency	Percent %
36-45	33	44.6%
26-35	24	32.4%
17-25	13	17.6%
46-55	4	5.4%

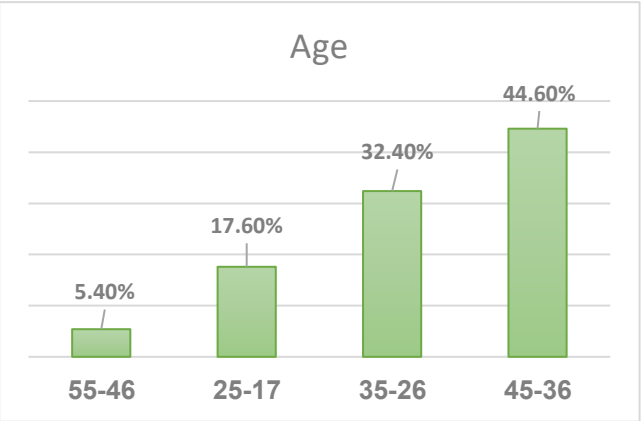

Figure (1) : Shows the percent % of ages at which anemia occurred.

2- Causes of injury

According to the findings of the present study, the most prevalent cause of anemia among the participants was a reduced appetite, which could be associated with pregnancy cravings or occur independently. Following closely was food scarcity, which could be attribute to various factors, including a low economic status. Additionally, some women reported a lack of knowledge about suitable dietary choices as a contributing factor, while others attributed their anemia to a history of recurrent abortions (Table: 3) (Figure 3).

Table (2): Shows the percent % of the causes of injury.

Causes	Frequency	Percent %
Lack of appetite due pica	38	51.4%
Unavailability of food	22	29.8%
Abortion	14	19%

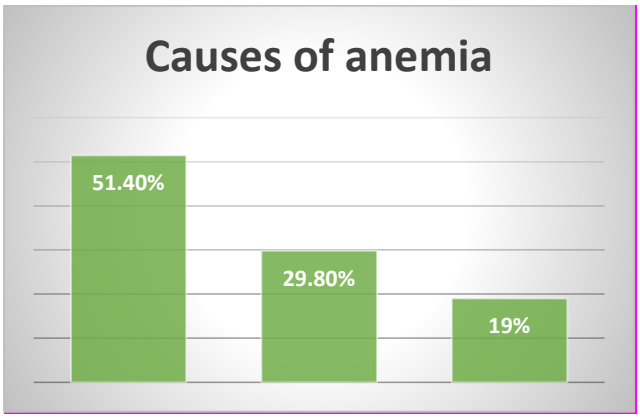

Figure (2): Shows the Percent % of the causes of anemia

3- Symptoms of the mother's disease

The study's findings revealed that the most common symptoms observed in pregnant women with anemia are headache, dizziness, shortness of breath, lack of concentration and lethargy, rapid heartbeat, blurred vision, bone pain, and loss of balance (Table 3), (Figure3).

Table (3): Symptoms of injury

Symptoms	Frequency	Percent %
Headache	53	% 71.6
Gid	46	% 62.2
Dyspnea	43	% 58.1
Inertia	37	% 50
palpitations	26	% 35.1
Blurred vision	24	% 32.4
Bone pain	19	%25.7
Loss of balance	10	% 13.5

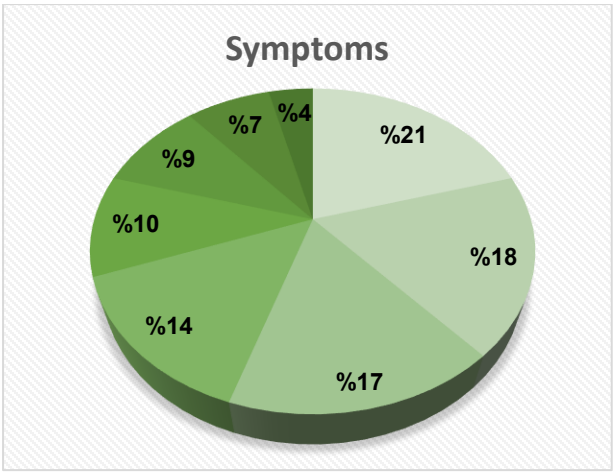

Figure (3): Shows the Percent % of disease symptoms

4- Abortion

The current study found that the incidence of miscarriage was low among pregnant women with anemia, which might be due to the fact that anemia does not target the important phase of pregnancy that causes miscarriage, namely the first trimester (the first three months) (Table: 4). (Figure: 4).

Table (4): Shows the incidence of abortion

Abortion	Frequency	Percent %
No	48	% 64.9
Yes	26	%35.1

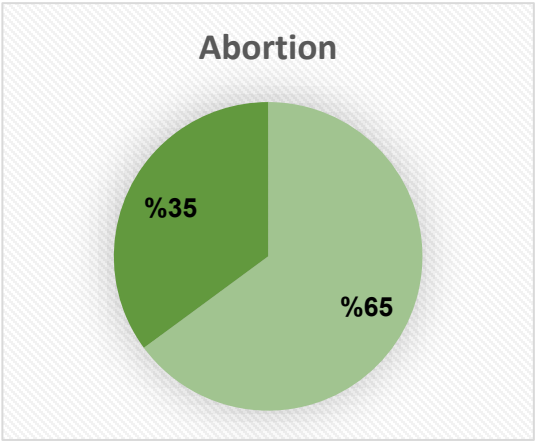

Figure (4): Shows the incidence of abortion

5- Diseases affecting newborns

The current study found that anemia in pregnant women resulted in anemia infection in infants, and other diseases including low weight, and anemia. (Table: 5). (Figure 5).

Table (5): Shows the incidence of congenital diseases

Disease	Frequency	Percent %
Jaundice	60	81.1%
Weight loss	53	71.6%

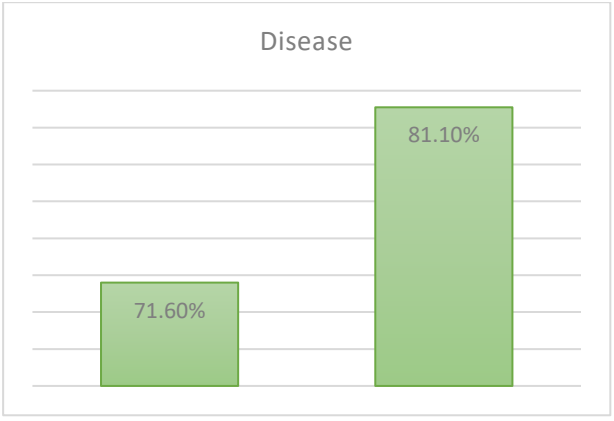

Figure (5): Shows the incidence of congenital diseases

6- Deformed births

The results of the current study did not indicate the occurrence of congenital malformations in the fetuses, and this may be due to several reasons. The disease, however, have not classified as a significant risk factor for congenital anomalies.

7- Stillbirths

The findings revealed that a handful of women had stillborn kids, although this was not a common occurrence. Pregnancy is a significant moment in a woman's life. This period is characterized by a variety of physiological, hormonal, metabolic, and psychological changes, and the pregnant woman does not adhere to a certain diet (Table: 6), (Figure: 6).

Table (6): Shows the Percent % of stillbirths

Stillbirths	Frequency	Percent %
No	49	%64.9
Yes	25	% 33.8

Figure (6): Shows the Percent % of stillbirths

4 Discussion

In the findings of the current study, it observed that individuals aged 17 to 25 had the lowest likelihood of infection, while those aged 36 to 45 exhibited the highest incidence of anemia. These findings align with previous studies (Hasswane *et al*., 2015) that investigated pregnant women in Morocco, where the age range of the study participants was 18 to 45 years. This variation may be attribute to differences in dietary customs across countries and regions. The underlying cause of anemia in pregnant women is an imbalance in hemoglobin and red blood cell production, often resulting from insufficient iron intake during pregnancy, a crucial nutrient for their formation. Alternatively, it may be link to the body's increased blood production during pregnancy to support the nutritional needs of both the mother and the developing child (Al-Mahjoubi *et al*., 2020). The study identified the most common causes of anemia, with the primary factor being a loss of appetite attributed to pregnancy cravings. This followed by food shortages resulting from poor economic conditions, a lack of information about suitable dietary choices, and anemia related to a history of recurrent abortions. These findings align with previous research (Zekarias *et al*., 2017). Studies suggest that anemia is often associated with a pregnant woman's history of abortions. The reason for this correlation is that blood loss during abortions depletes iron reserves, leading to anemia (Tadesse *et al*., 2017; Berhe *et al*., 2019), According to the study's findings, common symptoms in pregnant women with anemia include headaches, dizziness, shortness of breath, difficulty focusing, drowsiness, rapid heartbeat, blurred vision, bone pain, and loss of balance. These findings are consistent with the research conducted by other investigators (Gohar, 2014). The symptoms of anemia are contingent upon the severity of the condition, the rate at which it develops, and the individual's age. In cases of mild anemia, no noticeable symptoms or signs may emerge. However, as anemia progresses to a moderate stage, patients may experience a range of symptoms, including fatigue, shortness of breath, lethargy, headaches, pallor of the mucous membranes (including oropharyngeal mucosa), and muscle weakness (stoltzfus *et al*., 2007). Severe anemia can result in low blood pressure, renal failure, constipation, nausea appetite loss, elevated heart rate, and occasionally even death (Beers *et al*., 2006). These factors are not merely symptoms; they also indicate the severity and risks associated with the disease. Anemia poses a significant risk to expectant mothers, as one study has shown that it accounts for 20% of maternal fatalities, primarily due to insufficient blood reserves in mothers. Low hemoglobin levels in women, measuring less than 4 g/dL, can lead to partial heart failure, which may ultimately result in the death of the mother (Buseri *et al*., 2008). The recent study found that the occurrence of miscarriages was relatively low among pregnant women with anemia. This could be attributed to the fact that anemia does not typically affect the critical period for miscarriage, which is the first trimester (the first three months) of pregnancy. Most studies have shown that anemia tends to manifest more frequently during the third trimester (Al-Mehaisen *et al*, 2011; Seemal, 2012). This may be associated to the mother's increasing nutritional requirement against the backdrop of the mother's adverse eating habits in the first and second periods (Gatea *et al*., 2013). According to the current study, anemia in pregnant women can lead to

anemia in newborns and contribute to other related disorders, such as jaundice and low birth weight. Low birth weights can also result in complications affecting the digestive system, hormonal production, and metabolic processes (Allen, 2001). These metabolic changes may lead to a reduction in Glucose-6-Dehydrogenase (G6PD Phosphate), which is a cytoplasmic enzyme responsible for initiating the breakdown of hexose sugars in both the monophosphate hexose sugar pathway and the pentose phosphate pathway. These pathways result in the production of the reduced form of NADP, which plays a critical role in the synthesis and maintenance of reduced glutathione. Glutathione, in turn, serves to protect the red blood cell membrane from the toxic effects of oxidants. (Polin and Ditmar, 2001), The breakdown of red blood cells causes hemoglobin to decompose into the globin and heme parts, which change into bilirubin colors, and an increase in hemoglobin degradation may result in the onset of anemia symptoms (Robinson, 1990). In the absence of other causes of congenital jaundice, its level has evolved (Ho *et al*, 2007), G6PD enzyme deficiency has been demonstrated to produce acute and chronic hemolysis as well as neonatal hyperbilirubinemia (Dors *et al*., 2008). According to the findings, a small number of mothers experienced stillbirths, although this was not a common occurrence. Pregnancy is a significant phase in a woman's life, marked by a multitude of physiological, hormonal, metabolic, and psychological changes. Pregnant women do not necessarily adhere to a specific diet, but maternal malnutrition during pregnancy can contribute to fetal congenital abnormalities, preterm delivery, low birth weight, or even early mortality (Al-Dulaimi, 2016).

5 Conclusions

The current study identified the highest rates of infection in pregnant women between the mid-thirties and mid-forties. These infections were associated with symptoms that affected the pregnant mothers, including headaches, dizziness, shortness of breath, and difficulty concentrating. Additionally, there were cases of congenital malformations, although the cause of stillbirths was not determined.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Evaluation of Sunscreen Protection Factor Values (SPF) for some Aromatic Acids and their Salts of Mono- and Bivalent Metals by UV Spectrophotometer

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A B S T R A C T

This study aimed to determine the ultraviolet absorption for some carboxylic acids and their salts of mono- and bivalent metals as organic UV filters by UV spectrophotometer, as well as, calculate the values of the sun protection factor (SPF) for these compounds. The solutions of organic UV filters are subjected to absorbance measurements in the range of 290 to 320 nm, with five nm intervals, using the ultraviolet spectrometer. The experiments have been carried out in three different solvents: H_2O, MeOH, and EtOH. These salts included sodium benzoate, sodium salicylate, calcium benzoate, and calcium salicylate. The calculated sun protection factor (SPF) of these solutions was evaluated using the Mansur equation. All organic filters showed some sunlight protection properties. The best-calculated SPF values were 47.0 for salicylic acid, followed by the sodium salicylate salt at 40.3 and then the calcium salicylate salt at 39.7. These salicylic acid salts showed a high ability of UV absorbance compared to benzoic acid salts which showed SPF values of 11.5. This study presented organic UV filters with high SPF values and high solubility in polar solvents such as water and ethanol. Sodium and calcium salicylates would be recommended for use in the manufacture of sunscreen formulations.

1 Introduction

UV filters are chemical compounds that absorb ultraviolet radiation and minimize skin damage. UV filters can be classified into two groups according to their nature: inorganic UV filters and organic UV filters. Inorganic UV filters, also known as physical UV filters, are designed to reflect and diffuse rays to prevent them from penetrating the skin, while organic UV filters prevent rays from reaching the skin, both groups are classified as chemical UV filters (Dias-Cruz *et al*,. 2008; Chisvert & Salvador, 2007).

Minerals like titanium dioxide (TiO_2) and zinc oxide (ZnO) are widely used in sunscreen formulations such as broadband sunscreens that block UVA (290-320 nm) and UVB rays (320-400 nm) (Serpone *et al*., 2007).

Organic UV filters are compounds that contain organic compounds that have high absorption values in the ultraviolet range. These compounds typically consist of either single or multiple aromatic combinations and may be accompanied by double bond carbon links (Klimova *et al*., 2013; Li *et al*,. 2020). Figure 1 shows examples of organic filters that are commonly used in skin care products, such as sunscreens, to protect the skin from sunlight. Health encourages people to use sunscreen due to the harmful effects on the skin from excessive UV radiation, which mainly leads to skin cancer. Additionally, it has been found that a combination of UV filters is more effective than an individual ultraviolet radiation filter (Klimova *et al*., 2013; Li *et al*,. 2020).

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Organic UV filters must have terms that are heat stable, waterproof, non-toxic, easy to formulate, and safe for skin use (Nohynek & Schaefer, 2001). Three steps are outlined to explain how the skin absorbs UV rays (Klimova *et al*., 2015):

The entry of a substance into a particular pore or structure, such as the entry of a compound into the upper layer of the skin, is known as penetration.

Penetration is a layer-to-layer penetration, which is both a functional and a structural step and is the absorption of a substance into the lymphatic system.

Absorption is the process of absorbing through the skin and destroying the collagen layer of the skin.

The Sun Protection Factor (SPF) is a scale that measures the effectiveness of the sun's rays, helping to activate the natural defenses of skin protection from ultraviolet radiation (Brusie, 2020). The sun's protection factor's values indicate that the efficacy is determined by the absorption properties of each UV filtering material; since the skin is able to benefit from sunscreens because of their ability to absorb, reverse, and disperse UV rays; excessive exposure to the sun is closely linked to different skin types. (Catalano *et al*., 2020; Stiefel & Schwack, 2015).

Sunscreens are emulsions of chemical filters that contain water and oil, which can protect the skin from the harmful effects of UV radiation; the development of sunscreens is essential to keep the skin protected against UV rays, and preventing feedstock from interacting; sunscreen effectiveness can be achieved through the integration of soluble UVs into cosmetic formulations (Campos, Gaspar, 2006; Schulz, *et al*., 2002; Ngoc, *et al*., 2019; Mohiuddin, 2019).

Table (1) shows examples of allowance levels for some organic candidates in Canada, Australia, the European Union, and the United States (this is just a sample of countries) (Yarussi-King, 2017). Also, any organic filter needs to be licensed to allow it for example from the EU or FDA (Yarussi-King, 2017).

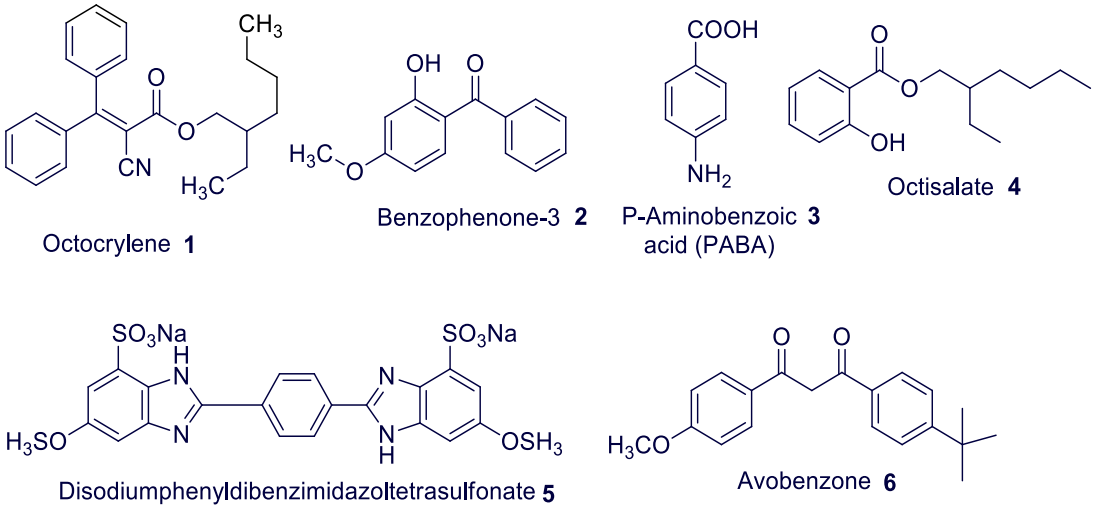

Figure (1): chemical structures of unknown organic UV filters (Klimova et al., 2013; Li et al,. 2020).

Table (1): The levels of organic candidates in four markets (Yarussi-King, 2017).

Sunscreen Active	European Union	United States	Australia	Canada
Octocrylene **1**	10% (as acid)	Up to 10%	10%	Up to 10%
Oxybenzone (Benzophenone-3) **2**	10%	Up to 6%	10%	Up to 6%
Aminobenzoic Acid (PABA) **3**	5%	Up to 15%	----------	Up to 15%
Octisalate (Etylhexyl Salicylate) **4**	5%	Up to 5%	5%	Up to 5%
Disodium phenyl dibenzimidazole tetrasulfonate **5**	10%	----------	10%	----------
Avobenzone (Butyl methoxydibenzoylmethane) **6**	5%	Up to 3%	5%	Up to 3%

2 Materials and Methods

2.1 Chemicals and Equipment

Sodium benzoate (98.00 - 100.50%) was bought from Riedel-de-Haen. Salicylic acid (99%) was purchased from ACORS. Ethanol (95%) and cyclohexane (99%) were obtained from BDH. Other chemicals were bought from Merk. Spectrophotometric determination of UV absorbance was carried out in a 1 cm path length cuvette (quartz) using a Jenway 6305 UV/visible spectrophotometer (single beam). Samples mixing were carried out using a vortex mixer (Bio Cote).

2.2 Calcium Benzoate Preparation

To a solution of 20 g (0.139 moL, 1.0 equiv) of sodium benzoate in distilled water (50 mL), the calcium chloride of 7.7 g (0.069 moL, 0.5 equiv) was slowly added at 25 ˚C with stirring. A white solid of insoluble calcium benzoate gradually precipitates is completed. The reaction mixture was stirred at 25 °C for an hour The mixture was then filtrated, washed with distilled water (25 mL), and dried to give calcium benzoate (8.8 mg, 23%) (Guang, 2021).

2.3 Calcium Salicylate Preparation

To a solution of 20 g (0.064 moL, 1.0 equiv) of sodium salicylate in distilled water (50 mL), the calcium chloride of 7.7 g (0.069 moL, 0.5 equiv) was slowly added slowly at25 ˚C with stirring. A white solid of insoluble calcium benzoate gradually precipitates is completed. The reaction mixture was stirred at 25 °C for an hour. The mixture was then filtrated, washed with distilled water (25 mL), and dried to give calcium salicylate (10.1 g, 26%) (Guang, 2021).

2.4 Sample Preparation and Measurement of UV Absorption

0.5 g of each sample was weighed, transferred to a 100 ml volumetric flask, diluted to volume with the suitable solvent (Table 2), followed by stirring using a vortex, and then filtered through filter paper. 5 ml of the stock solution of the filtrate was transferred to a 100 ml volumetric flask, diluted to volume with the suitable solvent (H_2O or EtOH), so that the sample concentration is 250 ppm.

Table (2): The solubility of samples in water and ethanol

No	Sample name	Suitable solvent
A.	Benzoic acid	Ethyl alcohol
B.	Salicylic acid	Ethyl alcohol
C.	Sodium benzoate	H_2O
D.	Sodium salicylate	H_2O
E.	Calcium benzoate	Ethyl alcohol
F.	Calcium salicylate	Ethyl alcohol
G.	Acetophenone	Methyl alcohol
H.	Benzophenone	Methyl alcohol

The absorbance of the prepared solutions of all samples (A-H) was measured by the UV spectrophotometer in the range of 290-320 nm using a 1 cm quartz cell, and this was done with an increase of 5 nm in each measurement. The measurement process was repeated twice for one wavelength, then the average absorbance of each sample was calculated as shown in Tables (3 and 4).

Table (3): Absorbance average's values of organic acids, mono- and bivalent metals salts, and ketones of organic acids

Wave length (nm)	Benzoic acid	Salicylic acid	Sodium benzoate	Sodium Salicylate	Calcium benzoate	Calcium Salicylate	Acetophenone	Benzophenone
290	0.166	1.588	1.116	1.378	0.359	1.367	0.873	1.447
295	0.029	2.179	0.658	1.924	0.417	1.822	0.462	1.204
300	0.016	2.510	0.513	2.238	0.407	2.241	0.264	0.747
305	0.009	2.598	0.382	2.313	0.356	2.346	0.152	0.356
310	0.006	2.641	0.266	2.168	0.238	2.262	0.123	0.217
315	0.004	2.529	0.186	1.393	0.121	1.569	0.114	0.205
320	0.004	2.799	0.132	0.691	0.045	0.832	0.420	0.217

3 Results

3.1 SPF Chemical Samples Account

The Mansur mathematical equation (1) is used to calculate the SPF values of the samples (A-H) (Mansur, *et al*, 1986; Sayre, *et al*, 1979; Dutra, *et al*, 2004).

$$\mathbf{SPF= CF \times \sum_{290nm}^{320nm} EE\ (\lambda) \times I\ (\lambda) \times ABS(\lambda)} \quad \mathbf{(1)}$$

Where: CF is the correction factor (=10); "EE", the erythemal effect of radiation at wavelength λ; "I", the intensity of the solar spectrum; and "ABS", the absorbance at wavelengths 290-320 nm. "EE", "I", and "ABS" are values obtained or applied for every wavelength (λ). The values for each of the [EE(λ) x I(λ)] are constants have been reported by the authors as normalized on the basis of the work by Sayre et. al., and are shown in Table 4 (Dutra, et al, 2004).

Table (4): The values of EE x I as they were determined by Sayre et al (1979).

Wave length (nm)	EE X I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180

4 Discussion

4.1. Research Strategy

The strategy of the study is focused on increasing the optical absorption of the organic filter by increasing the capacity of the exchange characteristic in π bonds that can increase the optical absorption of the organic filter because π bonds are responsible for the absorption of light in many organic molecules. When photons of light interact with a molecule, they can cause electrons in the π bonds to become excited and jump to higher energy levels. Then, the molecule is able to absorb light and generate an excited state.

The molecule's ability to absorb light can be enhanced by increasing the capacity of the exchange characteristic in π bonds, which increases its ability to accept and transfer electrons. This is because the exchange characteristic in π bonds is closely related to the conjugation of the molecule, which is the ability of electrons to delocalize across a series of adjacent atoms. When the exchange characteristic is elevated, conjugation is improved, resulting in a larger π electron cloud and, consequently, an enhanced absorption of light.

In addition, when using metal ions for salts, the solubility of this filter increases in very polar solvents such as water, methanol and ethanol. As shown in Figure (2), binary ions such as Ca^{+2} have the ability to double the size of the active organic filter, which allows it to have a good ability to absorb ultraviolet rays.

In general, the capacity of the exchange characteristic in π bonds can be enhanced to enhance its performance in applications such as solar cells or optical filters.

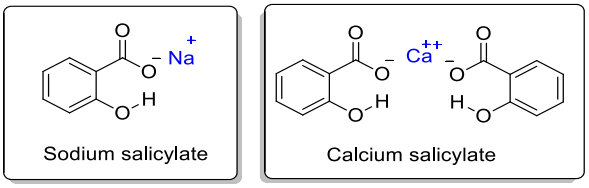

Figure (2): The structures of certain salts that are used.

4.2 Solubility Test of Samples of Organic Filters in Polar Solvents

Water was utilized as a solvent because it is the safest solvent to use on the skin. Table (5) shows that several attempts were made to obtain good solubility of the samples (A-H). The solvents are utilized because of their relative polarity, as revealed in Table 6.

Table (5): Solubility results for organic filters.

no	Organic UV filter	H_2O	MeOH	EtOH
A.	Benzoic acid	-	+	+
B.	Salicylic acid	-	+	+
C.	Sodium benzoate	+	+	+
D.	Sodium salicylate	+	+	+
E.	Calcium benzoate	-	+	+
F.	Calcium salicylate	-	+	+
G.	Acetophenone	-	+	+
H.	Benzophenone	-	+	+

Table (6): Used solvents arranged from the furthermost polar to the smallest polar.

Suitable solvent	Relative Polarity
H_2O	1.000
Methyl alcohol	0.762
Ethyl alcohol	0.654

4.3 SPF Values for Organic Filters

SPF values are numerical values that indicate the ability of a product to protect from the sun's rays. Thecalculated SPF values for organic filters are shown in Table (7).

Table (7): Calculated SPF values for organic filters.

no	Compound name	Calculated SPF values
A.	Benzoic acid	0.62
B.	Salicylic acid	47.04
C.	Sodium benzoate	11.5
D.	Calcium benzoate	11.5
E.	Sodium salicylate	40.3
F.	Calcium salicylate	39.7
G.	Acetophenone	7.5
H.	Benzophenone	7.9

In this study, the highest SPF value was found for salicylic acid, 47.04, followed by sodium salicylate (40.3), calcium salicylate (39.7), sodium benzoate (11.5), calcium benzoate (11.5), benzophenone (7.9), and acetophenone (7.8), as illustrated in Figure 1. These values show a high ability of salicylic acid and its salts to absorb UV rays compared to benzoic acid and its salts that showed lower SPF values. The reason of this low SPF values for benzoic acid salts may be attributed to the different structural construction between salicylic acid and benzoic acid. In the structure of salicylic acid, the presence of the electron-donating group (-OH) in the ortho position on aromatic ring increased the exchange capacity of π-bonds to give the compound a more reactive character than in benzoic acid, which lacks an electron-donating group on the aromatic ring (Figure 3).

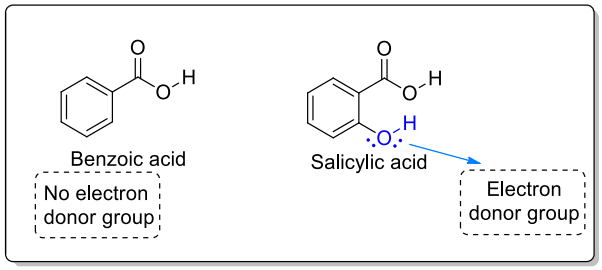

Figure (3): Comparison of the structural structures of the acids used.

The slight difference in the SPF values of the salicylic acid salts of sodium and calcium (E & F) may be attributed to the differences in the solvents (H_2O and EtOH) that effect on UV absorbency. On other hand, the SPF values did not change by changing the valence of the metal attached to the carboxyl group of the aromatic acid as observed from the obtained values. Thus, either mono- or bi-valent can be used in sunscreens formulations depending on which one is safer for the skin.

SPF values indicate the degree of protection a product offers against UVB rays, which are responsible for sunburn and skin cancer. The higher the SPF value, the greater the protection. It is recommended to use a sunscreen with an SPF value of at least 30. However, it is important to note that SPF values do not indicate protection against UVA rays, which can also cause skin damage. Therefore, it is important to use a broad-spectrum sunscreen that protects against both UVA and UVB rays.

Additionally, it is important to note that SPF values are not directly proportional to the amount of time aperson can spend in the sun without getting burned. Instead, the amount of time a person can spend in the sun without getting burned depends on a variety of factors, such as skin type, the intensity of the sun's rays, and the amount of sunscreen applied. Therefore, it is important to reapply sunscreen regularly and seek shade during peak sun hours to protect the skin from damage. Overall, this study suggests that salicylic acid and its salts may be more effective in providing protection against UV radiation compared to benzoic acid and its salts.

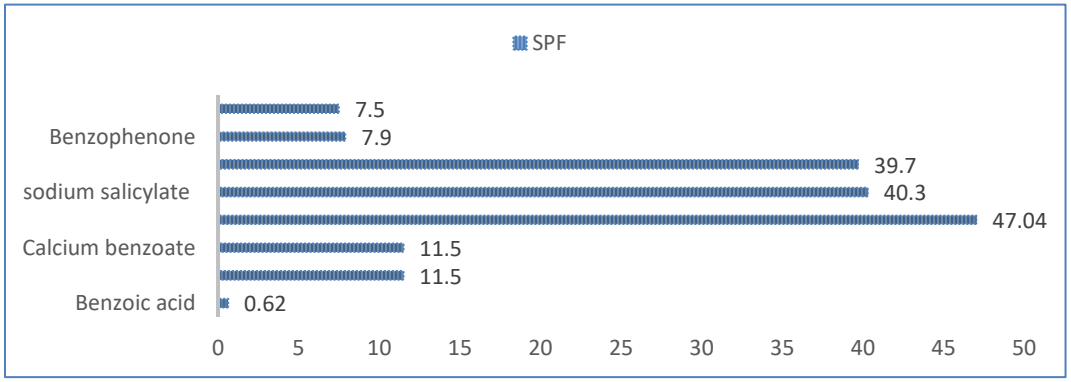

Figure (4): Comparison of the calculated SPF values for the samples used.

4.4 Comparison of the Obtained SPF Values with Previous Studies

Most of the previous studies that we conducted focused on natural extracts, whether from plant extracts or oils, instead of organic filters in our study, and did not exceed the SPF value 10 (Mosa, Makhlouf, 2019; Zayd, et al, 2019; Alfeetouri, Mosa, Jibreel, 2019; Mosa, Alsaady, Naser, 2022). Hence, we see that most of the organic filters give higher SPF values than filters extracted from plant samples, although salicylic acid was isolated from the bark of the willow tree and aspirin was prepared from it. In another study (Mbanga, et al, 2014), it was shown that the value of the SPF for some products containing organic filters (Table 7) written on the product and also calculated from Mansour's equation is much less than the values obtained from the filters under study.

Tables (7): calculated SPF values for organic filters in some formulations (Mbanga, *et al*, 2014).

no	Actives Ingredients	Labeled SPF	Calculated SPF
1	Avobenzene, Octylsalycilate, Titanium oxide, Terephthalylidène, Dicamphor sulfonic acid	15.00	15.24
2	benzophenone-3, octylmethoxycinnamate, octyldimethyl PABA	15.00	14.92

4.5 Uses of the Salts Under Study in Medicine and Food Industry

Sodium salicylate is used in medicine as an analgesic and antipyretic, also acts as a non-steroidal anti-inflammatory drug (NSAID) and induces apoptosis in cancer cells. However, calcium benzoate is used in the food industry as a preservative, its E number is E213; It is approved for use as a food additive in the European Union and other countries (Approved additives and E numbers, 2022). Sodium benzoate is widely used as a food preservative and pickling agent, and its E number is E211. Calcium salicylate is used as a preservative and also for the treatment of cancer (Cox, et al, 2021).

5 Conclusions

Measuring the SPF values of any organic filter is the best way to determine the effectiveness of sunscreen, so the higher the SPF value of the sunscreen, the greater the protection it provides against UV rays. In this study, the values of the SPF were measured for four aromatic salts added to their acids and samples of ketones, namely acetophenone and benzophenone, to compare them with the ability of the salts to absorb light.

This study presented organic filters that have a high ability to absorb in the UV region and gave high SPF values represented in salicylic acid, sodium salicylate salts, and calcium salicylate, and also distinguished from the rest of the filters by their ability to dissolve in highly polar solvents, which facilitates their use in skin care preparations to protect from Sun rays. Thus, this study contributed to the development of sunscreens to better protect against the sun's rays.

6 Recommendations

It is recommended to use salicylic acid salts in sunscreen products as organic filters which have a high ability to filter UV rays and prevent their penetration into the skin; as well as these salts dissolve in water which is the safest solvent to use on the skin. Further research is also recommended in the field of organic filters that are safe and easily soluble in polar solvents such as water.

Contrast of Interest: The authors declare that they have/ have no contrast of interest associated with this manuscript.

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Impact of CO_2 Concentration on Indoor Air Quality in Various Schools and Colleges in Baniwaleed City

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A B S T R A C T

Indoor air pollution has been known since ancient times, but the types of indoor air pollutants differ with the presence of modern buildings. Millions of people die each year as a result of the serious threat that indoor air pollution poses to human health. Numerous pollutants can cause indoor air pollution; therefore, it's crucial to identify their primary sources and concentrations and develop plans for enhancing and controlling the quality of the air indoors. Air-conditioned and tightly closed, especially since a person spends more than 80% of his day in closed environments. The current study included measuring the concentration of carbon dioxide inside some schools and colleges in Baniwaleed city to ensure air quality. Two primary schools, four secondary schools, and two colleges were selected. Measurements were taken twice, the first in November and the second in December. A HT 2000 CO_2 meter was used for measurement, located in the laboratories of the Faculty of Science, Baniwaleed University, Department of Geology and Environmental Sciences. The highest reading was recorded at 2221 ppm at Sana Muhaidli School for Secondary Education in the month of December. The second highest reading recorded in the College of Engineering was 2005 ppm, and it was in the month of December as well. There were some readings within the permissible limits (less than 1000 ppm) and some readings higher than the permissible limit.

1 Introduction

Most of the time that people are awake is spent working or studying. As a result, facility managers and building operating engineers are placing a high importance on maintaining sufficient indoor air quality (IAQ) in workplaces and educational institutions. In order to maintain adequate indoor air quality, outside air is essential for dilution of indoor air contaminants and their removal from our buildings, along with moisture and odors.

Both the combustion process and the metabolic process in living things produce carbon dioxide (CO_2). Since human metabolism produces carbon dioxide, measurements of its levels inside buildings are frequently used to determine if the area is receiving enough fresh air. Carbon dioxide is one of the pollutants of indoor air that is most frequently discussed. Despite not being considered a dangerous air pollutant, carbon dioxide replaces oxygen in enclosed spaces, lowering the quality of indoor air (Owen, 2009). According to the World Health Organization (WHO), 3.8 million people die each year as a result of indoor air pollution worldwide.

High amounts of carbon dioxide can result in nausea, dizziness, and vomiting, while moderate to high concentrations might result in headaches and exhaustion. Extreme concentrations can cause loss of consciousness.

Fresh air should be brought into the space to prevent or lower high carbon dioxide concentrations in a building or room. According to past studies, inadequate

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classroom ventilation might have a negative impact on both teachers' and students' health. In Baniwaleed, schools frequently have natural ventilation. A classroom air quality test determines the level of air pollution based on their high occupancy, allowing for the execution of corrective measures. Carbon dioxide (CO_2) is produced by both the metabolic and combustion processes in living things.

Since human metabolism produces carbon dioxide, measurements of its levels inside a structure are frequently used to determine if the area is receiving enough fresh air. High amounts of carbon dioxide can result in nausea, dizziness, and vomiting, while moderate to high concentrations might result in headaches and exhaustion. CO_2 content has frequently been employed as a gauge for ventilation system effectiveness (Coley et al, 2007). At least two factors contribute to the importance of ventilation in schools. First, there's the issue of air quality and how it affects people's health and productivity. It is also a channel for heat loss. There is conflicting information about the impact of low ventilation rates on productivity and health (Myhrvold et al, 1996).

It is quite simple to measure the amount of CO_2 gas present in a given sample of air (Mahyuddin and Awbi, 2012). Since outside CO_2 directly affects interior concentrations, it is necessary to monitor outdoor CO_2 levels when evaluating indoor concentrations. The typical range of CO_2 content in outdoor air is 350 to 450 ppm (Jones, 1999). The amount of cubic feet of air per minute (cfm) provided per person can be used to gauge how well a ventilation system is functioning. Alternately, indoor CO_2 concentrations can be used to gauge the effectiveness of ventilation. For instance, a 1000 ppm CO_2 level corresponds to 15 cubic feet of fresh air per person per minute (Ellenbecker, 1992).

The ASHRAE advises against indoor CO2 concentrations that are more than 700 ppm above outdoor ambient values. For businesses and schools, ASHRAE recommends that indoor CO2 levels be fewer than 800 ppm and 1000 ppm, respectively (ASHRAE, 1992).

CO_2 concentrations in indoor air that are greater than 1000 ppm are regarded by the National Institute for Occupational Safety and Health (NIOSH) as a sign of insufficient ventilation (Sireesha, 2017). The key measurements of occupants' thermal comfort are the indoor air temperature (T) and relative humidity (RH) (Kavgic, et al, 2012). High RH can result in condensation and the growth of mold, both of which are harmful to occupants and young children (Abuku et al, 2009).

High carbon dioxide (CO_2) levels could be dangerous for those living in houses, flats, classrooms, and workplaces, including office buildings. Many people struggle with the uncertainty surrounding what high CO_2 levels are, how to measure them, and how to mitigate them.

Organizations like the Occupational Safety and Health Administration, the Center for Disease Control, and the U.S. Green Building Council are working to provide enough information about the significance of monitoring CO_2 levels indoors and what the potential long-term effects of exposure are for people exposed to higher amounts of CO_2.

Many individuals tend to be surprised when it comes to understanding the importance of carbon dioxide monitoring and recognizing the direct impact high CO_2 concentrations can have on their overall well-being, health, and cognitive skills.

Technically, anything beyond the "safe" CO_2 levels indoors denotes a fresh air flow issue rather than excessive CO_2 levels specifically. A number of studies over the years, however, have discovered a direct link between elevated CO_2 levels indoors (caused by a lack of fresh air) and elevated levels of mold, dust, germs, and viruses in the air. Additionally, elevated CO_2 levels are linked to decreased cognitive function and an increase in sleepiness.

The aim of this study is to measure the percentage of carbon dioxide and the indoor air temperature (T) in order to ascertain the indoor air quality in a number of schools and colleges in Baniwaleed city (primary two schools, secondary four schools, and two colleges). The readings were taken over two periods, November and December of 2022, and Table 1 shows the details of the selected schools and colleges. All schools and colleges are naturally ventilated, and air can continuously enter and exit the classrooms through doors, windows, cracks, and other openings. Schools and colleges vary in age, building and size. Each designated classroom had one outer wall, one side in contact with an interior corridor, and the other two walls in contact with adjacent classrooms.

2 Methodology

2.1 Sampling Site Description and Measurement Period

The object of this paper is the analysis of CO_2 and indoor air temperature (T) and their variability in two basic education schools, four Secondary schools, and two colleges in Baniwaleed city. For all selected schools and colleges two sampling campaigns were performed during the heating season (November and December). The main parameters of the selected schools are given in Table. 1.

Table 1: shows the details of the selected schools.

no	name
1	Hafez Al-Madani School
2	Al-Barq Al-Khatif School
3	Al Quds School
4	Sanaa Muhaidly School
5	Badr Al-Kubra School
6	Almasira Alkubra School
7	College of Engineering
8	College of Medical Technology

2.2 The Device Used for Measurement

The HT 2000 CO_2 meter is located in the laboratories of the College of Science, Department of Geology and Environmental Sciences. Photo No. 1 shows the device.

Photo 1: HT 2000 CO_2 meter.

3 Results and Discussion

Indoor air quality (IAQ) in schools is an important public health concern. Especially children should be taken care of because they are more exposed to air pollution than adults. Although carbon dioxide is not considered a toxic air pollutant, its concentrations indoors can have a negative impact on users. The permissible concentration of carbon dioxide in confined spaces is 1000 ppm, these minimum health requirements are recommended by the European Office and the World Health Organization.

3.1 Al-Barq Secondary School

The average concentration of carbon dioxide in Al-Barq Al-Khatif Secondary School was 626 ppm during the month of November and 701 ppm during December, and the highest reading was recorded in November, 850 ppm, at a temperature of 21 °C and a humidity of 57%. It is within the permissible rate of carbon dioxide concentration in closed places, which indicates the presence of good ventilation in the classrooms of this school. Tables 3 and 4 and Figures 1–4 show averages of carbon dioxide concentration and temperature, as well as the highest and lowest readings recorded during November and December.

3.2 Hafez Al-Madani School

The average concentration of carbon dioxide in Hafez Al-Madani School for Secondary Education was 1179 ppm during the month of November and 1053 ppm during December, and the highest reading recorded in November was 1372 ppm at a temperature of 22 °C, which is higher than the permissible rate of carbon dioxide concentration in closed places, which indicates the absence of good ventilation in the classrooms. This school and classrooms need to be ventilated. Tables 2 and 3 and Figures 1–4 show averages of carbon dioxide concentration and temperature, as well as the highest and lowest readings recorded during November and December.

3.3 Al Quds School

The average concentration of carbon dioxide in Al Quds School for Secondary Education for Girls was 921 ppm during the month of November and 728 ppm during December, and the highest reading recorded in December was 1457 ppm at a temperature of 23 °C. It is higher than the allowed rate of carbon dioxide concentration in enclosed spaces, and it was recorded in one classroom while the other classrooms had less than 1000 ppm, meaning that care must be taken to ensure good ventilation in all classrooms inside the school. Tables 2 and 3 and Figures 1–4 show averages of carbon dioxide concentration and temperature, as well as the highest and lowest readings recorded during November and December.

3.4 Sanaa Muhaidly School

The average concentration of carbon dioxide in Sanaa Muhaidly Secondary School was 755 ppm during the month of November and 1477 ppm during December, and the highest reading was recorded in December at 2221 ppm, at a temperature of 19 °C. It is higher than the allowed rate of carbon dioxide concentration in enclosed spaces, meaning that care must be taken to ensure good ventilation in all classrooms inside the school. Tables 2 and 3 and Figures 1–4 show averages of carbon dioxide concentration and temperature, as well as the highest and lowest readings recorded during November and December.

3.5 Badr Al-Kubra School

The average concentration of carbon dioxide in Badr Al-Kubra School for Basic Education was 728 ppm during the month of November and 1068 ppm during December, and the highest reading was recorded in December, 1418 ppm, at a temperature of 17 °C, which is higher than the allowed rate of carbon dioxide concentration in closed places. Most of the readings for the month of December were high, exceeding the permissible limits, while the readings in November had readings within the permissible limits (less than 1000 ppm). Therefore, all classrooms must be ventilated inside the school. Tables 2 and 3 and Figures 1–4 show averages of carbon dioxide concentrations and temperatures, classroom size, the highest reading recorded, and the lowest reading recorded during November and December.

3.6 Almasira Alkubra School

The average concentration of carbon dioxide recorded in Al-Masirah Al-Kubra School for Basic Education was 724 ppm during the month of November and 970 ppm during December, and the highest reading was recorded in December, 1329 ppm, at a temperature of 17 °C, which is higher than the allowed rate of carbon dioxide concentration in closed places. There were only a few readings during the month of December that exceeded the permissible limits, while those during the month of November were mostly within the permissible limits (less than 1000 ppm). It is recommended that all classrooms be ventilated inside the school. Tables 2 and 3 and Figures 1–4 show averages of carbon dioxide concentrations and temperatures, classroom size, the highest reading recorded, and the lowest reading recorded during November and December.

3.7 College of Engineering

The average concentration of carbon dioxide in the College of Engineering at Baniwaleed University was 1405 ppm during the month of November and 1980 ppm during December, and the highest reading was recorded in December, 2005 ppm, at a temperature of 18 °C, which is higher than the allowed rate of carbon dioxide concentration in closed places, and most of the readings for the month of December were higher than the allowed rate, which indicates a problem with ventilation. Therefore, we recommend ventilating the halls between lectures for 10 minutes and taking new measurements of carbon dioxide concentration. If the concentration remains high, mechanical ventilation must be used, and suction fans should be used to exchange the air. Tables 2 and 3 and Figures 1–4 show averages of carbon dioxide concentrations and temperatures, classroom size, the highest reading recorded, and the lowest reading recorded during November and December.

3.8 College of Medical Technology

The average concentration of carbon dioxide in the College of Technology at Baniwaleed University was 964 ppm during the month of November and 790 ppm during December, and the highest reading was recorded in November, 1080 ppm, at a temperature of 23 °C, which is higher than the allowed rate of carbon dioxide concentration in closed places, while most of the readings were within the permissible rate, except for one reading in November, which recorded 1080 ppm.

We recommend good ventilation after each lecture by opening windows and doors to allow air to circulate inside the classrooms. Tables 2 and 3 and Figures 1–4 show averages of carbon dioxide concentrations and temperatures, classroom size, the highest reading recorded, and the lowest reading recorded during November and December.

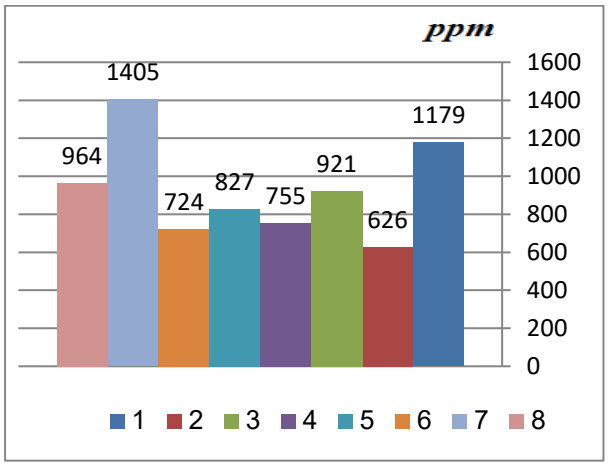

Figure 1: Average carbon dioxide concentrations (ppm) during the month of November

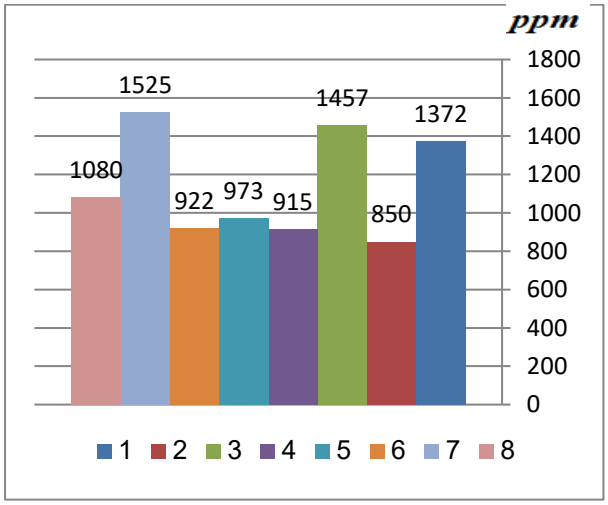

Figure 2: The highest concentrations of carbon dioxide (ppm) during the month of November

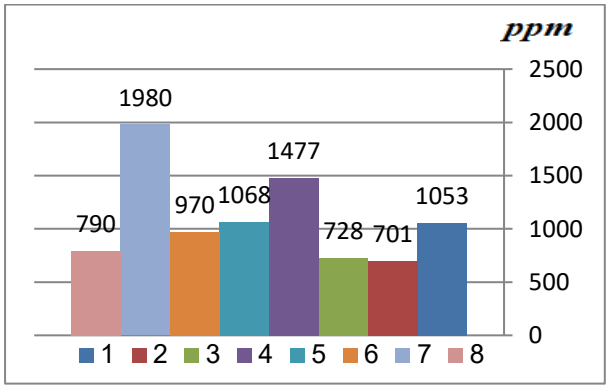

Figure 3: Average carbon dioxide concentrations (ppm) during the month of December

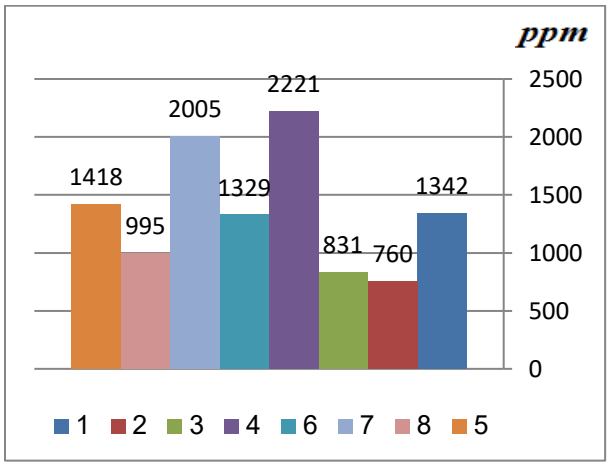

Figure 4: the highest concentrations of carbon dioxide (ppm) during the month of December

Table 2: show averages of carbon dioxide concentrations, temperatures, classroom size, the highest reading recorded and the lowest reading recorded during November

no	max ppm	min ppm	temperature ° C	size m^2	CO_2 ppm	Student
1	1372	820	22	35	1179	27
2	850	512	21	35	626	28
3	1457	643	23	30	921	33
4	915	532	25	35	755	36
5	973	594	19	30	827	21
6	922	516	19	30	724	27
7	1525	1289	27	120	1405	33
8	1080	574	23	80	964	35

Table 3: show averages of carbon dioxide concentrations, temperatures, classroom size, the highest reading recorded and the lowest reading recorded during December.

no	max ppm	min ppm	temperature ° C	size m^2	CO_2 ppm	Student
1	1342	763	18	35	1053	27
2	760	582	19	35	701	28
3	831	647	23	30	728	33
4	2221	1153	19	35	1477	36
5	1418	785	17	30	1068	23
6	1329	684	17	30	970	27
7	2005	1290	18	120	1980	74
8	995	586	18	80	790	35

4 Conclusion

As a result of regular metabolic processes, people create and exhale carbon dioxide (CO_2), which is why indoor CO_2 concentrations are higher than outdoor CO_2 concentrations in occupied buildings. Higher CO_2 levels when our bodies go through their natural cycles might be blamed for unpleasant sensations, fatigue, lack of concentration, and even nausea. This may result in sick building syndrome indoors. In fact, elevated CO_2 levels indoors may potentially exacerbate a number of the sick building syndrome symptoms.

The amount of CO_2 in school classrooms has a significant impact on indoor air quality. Low indoor air quality appears to be an issue in both newly constructed buildings and older structures in naturally ventilated schools. The aim of this study is to measure the percentage of carbon dioxide and the indoor air temperature (T) in order to ascertain the indoor air quality in a number of schools and colleges in Baniwaleed city (primary two schools, secondary four schools and two colleges). The readings were taken over two periods during the heating season: November and December of 2022. All schools and colleges are naturally ventilated, and air can continuously enter and exit the classrooms through doors, windows, cracks, and other openings. Schools and colleges vary in age, building, and size. Each designated classroom had one outer wall, one side in contact with an interior corridor and the other two walls in contact with adjacent classrooms. Results obtained in this study confirm that the levels of carbon dioxide concentrations in some schools and colleges in Baniwaleed city are higher than the permissible limits, which will affect the health of students and thus their academic achievement. The highest reading was recorded at 2221 ppm at Sana Muhaidli School for Secondary Education in the month of December. The second highest reading recorded in the College of Engineering was 2005 ppm, and it was in the month of December as well. There were some readings within the permissible limits (less than 1000 ppm) and some readings higher than the permissible limit. The study also made the following observations:

- The amount of time between classes is insufficient to allow the CO_2 concentration to decrease sufficiently to reach the outside level.
- High CO_2 concentration levels in the classrooms and poor air exchange rates in naturally ventilated schools are mostly caused by insufficient ventilation and the frequency and length of window openings.
- The findings of this study further highlights the requirement for a management approach for monitoring CO_2 concentration levels and air exchange rates in educational facilities.

Overall, by keeping an eye on CO_2 levels, one can safeguard tenant health, boost productivity, control costs, adhere to rules, and uphold a good reputation. The prevalence and effects of sick building syndrome can be reduced by establishing healthier and cozier indoor settings, which will help both people and businesses.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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In vitro phytochemical screening, Antioxidant and Anti-platelet Activities of Libyan *Arbutus pavarii* Extract

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A B S T R A C T

This study was conducted to investigate the active compound like alkaloid and phenolic compound in leaves and fruit extracts of *Arbutus pavarii* compound and to determine the potential effect of methanol extract of *Arbutus pavarii* to improve antioxidant properties activity in a dose-dependent manner with good IC50 values. The antioxidant effect of *Arbutus pavarii* was investigated by scavenger effect using 1, 1-diphenyl-2 picrylhydrazyl (DPPH). The researchers observe the possibility of *Arbutus pavarii* extract to prevent human platelets aggregation. The effect of *Arbutus pavarii extract* related to its active compounds including polyphenols which able to reduce the scavenge of the hydroxyl radicals in human body leading prevent dysfunction pathway, the mechanism underlying such effects related to a reduce the vascular formation of Reactive oxygen species (ROS). The present findings indicated that *Arbutus pavarii* has antioxidant effects and the ability to prevent human platelets aggregation. These results could be indicator to improve the verity cells dysfunction in human body, then improve the function of cardiovascular and also to reduce pathological arterial wall. Further suggest that *Arbutus pavarii* might be an interesting candidate as a good food therapeutic.

1 Introduction

Arbutus pavarii Pampan (Family Ericaceae) is a deciduous or average shrub, tree reaching up to 3 meters distributed in the West area of Libya (El-Gabel El-Akhdar). The bark has faint, smooth, and reddish, peeling. The fruit takes around 8 months to ripen, and they are spherical and warty, and turn from yellow to orange to scarlet as the autumn progresses. The strawberry fruits are edible directly as fruits or can be made into jam, but the taste is somewhat insipid (Elshatshat, 2009). Several epidemiological studies have indicated that regular intake of beverages rich in polyphenols is associated with a reduced risk of several diseases including cardiovascular diseases (Di Castelnuovo *et al*., 2002; Kuriyama *et al*., 2006). The beneficial effect has been attributed at least partly to their high polyphenol such as procyanidins and anthocyanins (Corder *et al*., 2006 ; Auger *et al*., 2010;). Moreover, the effect of anthocyanins on oxidative stress may involve its ability to inhibit Reactive Oxygenase Species (ROS) formation possibly also by regulating the expression of several pro- and antioxidant enzymes. Moreover, this effect may be attributed at least to indirect protection by activating endogenous defense systems and by modulating cellular signaling processes.

Polyphenols might protect the cardiovascular system by a variety of actions, including their ability to improve the lipid profile by their antioxidant properties, which will prevent the degradation of nitric oxide (NO) by superoxide anions (Frankel *et al*., 1993; Ndiaye *et al*., 2005; Sarr *et al*., 2006). The antioxidant properties of the phenolic compounds were reported as a scavenger of the

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1, 1-diphenyl-2 picrylhydrazyl (DPPH) (Baratto *et al*., 2003; Benhammou *et al*., 2007; Gardeli *et al*., 2007). It's also shown that the presence of Gallic acid and its derivative, like 1, 2, 3, 4, 6-pentagalloyl glucose (ply) putative role agents lipid peroxidation induced by H_2O_2 in K562 cell line (Abdelwahed *et al*., 2007). Several epidemiological studies and randomized control trials have indicated an inverse relationship between the dietary intakes of beverages rich in polyphenols on platelet activity. The primary objective of this study is to investigate the active compound in extract of *Arbutus pavarii*, and the effect of polyphenol from *Arbutus pavarii* as a potent antioxidant.

The second objective is to investigate in-vitro the extracts of *Arbutus pavarii* as antiplatelets effect lading to reduce human platelets aggregation.

2 Materials and Methods

2.1 Plant material Preparation

The leaves and fruits of *Arbutus pavarii* were collected from western area in Libya and air-dried at room temperature and reduced to fine powder by milling then stored in a dry place in airtight container.

2.2 Preliminary Phytochemical Screening

The leaves and fruits of *Arbutus pavarii* was prepared for evaluation of phytochemical constituent as following (sofowora, 1993; Trease and Evans, 2002)

2.3 Plant Material Extraction

The dried plant powder (1000 g) and 500 g of fruit was extracted by using cold maceration in methanol for 72 h. Then extraction was repeated three times, and methanol extract was collected and concentrated by rotary evaporator at 60 °C, under reduced pressure. The extracts were weighted and stored in a refrigerator at 4 °C (sofowora, 1993).

Test of alkaloids

Few mgs of each extract (leaves and fruits) was separately stirred with 1% HCl on a water bath for 5 min and filtered. These filtrates were treated with few drops of Dragendorff's reagent (Potassium bismuth iodide solution), Mayer's reagent (Potassium mercuric iodide solution), and Wagner's reagent (Potassium iodide and iodine). A formation of precipitate indicates the presence of alkaloids (Trease and Evans, 2002).

Test of Tannins

Each extract(leaves and fruits) (0.5mg) was separately stirred with distilled water (10 mL) and then filtered. A few drops of 5% ferric chloride were then added. Black or blue-green coloration or precipitate was taken as a positive result for the presence of tannins (Trease and Evans, 2002)

Test for Saponins

Each of plant extracts(leaves and fruits) (0.5) was separately shaken with distilled water (10 mL) in a test tube then heated in warm water bath for 5 min. The formation of a froth shows the presence of saponins (sofowora, 1993).

Test of Coumarins

One drop of extracts was dissolved in NaOH, then spotted on Wattman's filter paper and detected under UV light (366nm), blue fluorescence spots indicated the presence of coumarins (sofowora, 1993).

Test of Flavonoids

The extract (leaves and fruits)was treated with magnesium turnings and one drops of concentrated HCL. The formation of red or pink color showed the presence of flavonoid (sofowora, 1993).

Test of Anthraquinone Glycoside (Borntrager's Test)

The extract (leaves and fruits) solution was adding 5% H_2SO_4 (1 mL). The mixture was boiled in a water bath for 10 min and then filtered. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then the lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose pink to red color of the ammoniacal layer as indication of anthraquinone glycosides (sofowora, 1993).

The MTT Assay

The MTT assay is a colorimetric assay for assessing cell metabolic activity. Cell viability was performed using HeLa cell line. Cells were treated with different concentrations of plants extracts including (0.1, 0.3, 1.0 mg/ml).100 µL/well in a 96-well plate. MTT Reagent was adding to each well at a 1:10 ratio, after incubation for 2-4 hours or overnight at 37°C. Cells well are mentoring under the microscope for the presence of a purple precipitate. 100 µL of Detergent Solution for every 10 µl of MTT Reagent added to each well (100 µl

/well for a 96-well plate or 250 µl /well for a 24-well plate). Gently mix the solution by pipetting. Cover the plate to protect it from light and incubate in the dark for 2-4 hours at room temperature and measure the absorbance in each well at 570 nm in a microtiter plate reader. (Mosmann, 1983).

DPPH(1-diphenyl-2-picrylhydrazyl) radical scavenging effect

The antioxidant properties of *Arbutus pavarii* was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. Methanol extract of solutions of the test samples at various concentrations ((20-100µg/mL).) was added to a solution of DPPH in methanol (0.001% w/v) in was taken into the cuvettes then *Arbutus pavarii* extracts were added followed by serial dilutions (1 mg to 500 mg) to every cuvette so that the final volume was 1 mL and after 30 min, the absorbance was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a reference standard and was dissolved in methanol to make the stock solution with a concentration of 1 mg/mL. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. Blank was prepared with 99% methanol.

$$\text{percentage of scavenging} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test}}{sample\ x100}$$

Absorbance of the control

The inhibition curve was plotted for duplicate experiments and represented as % of mean inhibition ± standard deviation. IC50 value was determined from the graph obtained by using standard ascorbic acid following the formula "y=mx+c" from the slope of the graph. The IC50 represents the concentration where 50% inhibition of the DPPH radical is obtained.

seeds) may be used as a source of antioxidants for pharmacological and food preparations which is very well evidenced by the present work. The antioxidant activities in terms of IC50 of C. maxima extracts were summarized in *Arbutus pavarii* .

Platelet aggregation

Platelet aggregation well measured by Born's method (Born *et al*., 1971) using a dual channel automatic optical aggrego meter (560, Chrono-log Co.). Human platelet-rich plasma (PRP) was incubated at 37°C for 2 min in the aggrego meter with stirring at 1,000 rpm and exposed to various concentrations (0.1, 0.3, or 1 µg /ml) of plants for 3 min. After incubation, platelet aggregation was induced by addition of collagen (20 µg/ml) And use aspirin to compare with the extract as an anticoagulant. The resulting aggregation was measured the change in light.

3 Results

3.1 Preliminary phytochemical screening

We performed phytochemical screening using different chemicals and solvent to investigate different active compound in experimental plant extract. our results indicate the presence of coumarin, flavonoid, saponins and, these results indicator to interesting plant for antioxidant properties (table1).

Table (1): Preliminary phytochemical screening

Extracts	IC50 µg/ml
Leaves	4.2±0.22
Fruit	4.3±0.24
Ascorbic Acid	51.3±0.005

The cytotoxicity activity of methanolic extract was detected in HELA (cervical carcinoma cell line). Different concentration of plant extract was added (12.5 to 100.0 mg/ml up to 72 hours. Our results showed no cytotoxicity was detected even at concentrations up to 100 µg/mL (Figure1).

Cytotoxic assay

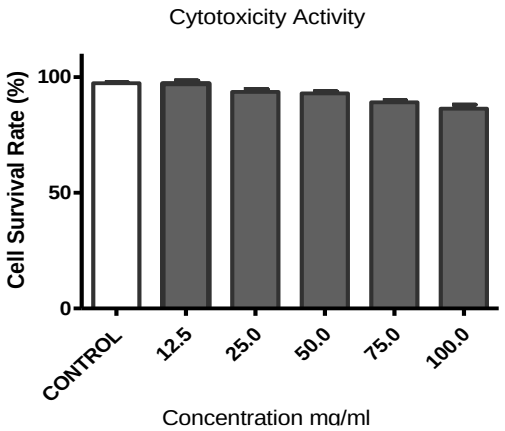

Figure (1): Cytotoxicity assay following treatment of HELA (cervical carcinoma cell line)

DPPH free radical scavenging activity assay

we calculate the percentage of antioxidant activity versus extract concentrations (IC50) values, which calculated from the linear regression of the percentage of antioxidant activity versus extracts concentrations. Both leaves and fruits extracts as shown in table (2).

Table (2): antioxidant activity of leaves and fruit

Photochemical	Crud Methanolic
Alkaloids	- Ve
Coumarin	++ Ve
Glycoside	- Ve
Falvonoid	+ Ve
Saponins	+ Ve
Tannin	+ Ve

Our results indicate that the *Arbutus pavarii* extract showing high antioxidant activities, these results may have related to the therapeutic effects. The percentage of inhibition shown respectfully concentration dependent effect in both the extract for leaves and fruits- (Figure2 and Figure3)

Antioxidant Activity

In our study, we investigated the possibility that the free radical scavenging action of *Arbutus pavarii* extract. Bot leaves and fruit of methanolic extract have potential antioxidant reduce inducing free radical, suggests that *Arbutus pavarii* has antioxidant activities that could be candidate for future therapeutic

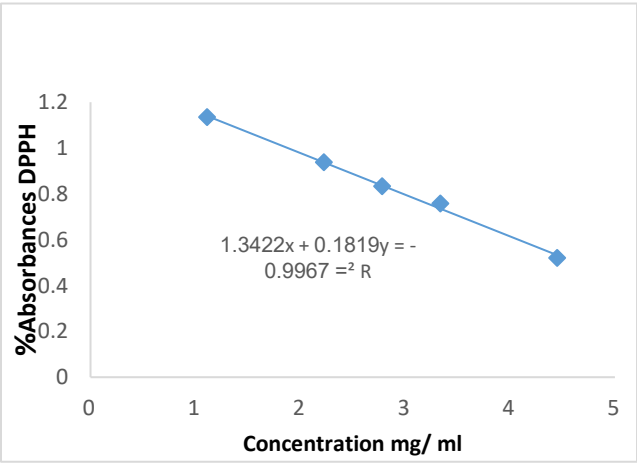

Figure (2): DPPH free radical scavenging activity of Arbutus pavarii leaves methanol extract

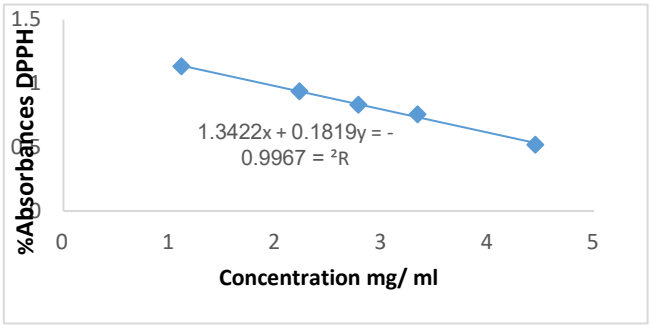

Figure (3): DPPH free radical scavenging activity of Arbutus pavarii fruit methanol extract

Effect of *Arbutus pavarii* on platelets aggregation induced by collagen.

Human platelets were pre-incubated with various concentrations of *Arbutus pavarii* (0.1, 0.3, or 1 mg/ml), and then exposed to collagen (20 µg/ml). As shown in Figure (4) strongly inhibited the platelet aggregation induced by collagen at 1 mg/ml mg/ml. The inhibitory effect on collagen -induced aggregation was more pronounced and shown dose-dependent manner. In addition, the inhibitory effect of on extract-induced (leaves and fruits) platelet aggregation was comparable to aspirin (1.0 ± 0.5 and 1.0 ± 0.7 mg/ml, respectively).

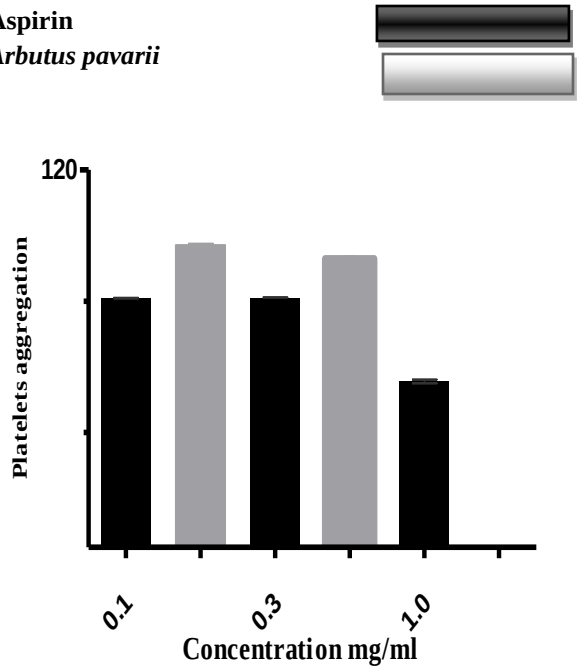

Figure (4): Effect of *Arbutus pavarii* on platelet aggregation by ADP.

Representative platelet aggregation profile in collagen treated platelets in absence or presence of aspirin (1 mg/ml), *Arbutus pavarii* (1 mg/ml) (A) and cumulative result in dose dependent manner (B). All data represent means ± S.D. (n=3). *$p < 0.05$, compared with untreated control

4 Discussion

In primary results the extract of *Arbutus pavarii* shows no potent cytotoxic activity on HELA (cervical carcinoma cell line). In second part the result shown that the *Arbutus pavarii* plant rich in active compound like alkaloids, flavonoid, coumarin, sponins ,and glycoside (Ehsani-Moghaddam *et al*., 2006),these results were compatible with previous studies indicate that *Arbutus pavarii* rich in such active compound (Navindra *et al*., 2006) in third part , we do the antioxidant properties , and our results indicate that the plant has antioxidant effect, these results was improvement by the scavenger of DPPH reagent and IC50 value which indicate dependent effect with increase the concentration of plant extract, these results were compatible with other results from different studies (Navindra P. Seeram et al., 2006) moreover theses effect may have related to the content of polyphenols, further (support an important role of polyphenols is important in preventing the cardiovascular diseases, inflammatory diseases and cancer. Polyphenols are well known not only to have antioxidant properties due to their structure but also to have the ability to modulate the activity of different kinds of enzymes and cell receptors.

As several compounds with antioxidant properties have demonstrated a capacity to reduce the harmful effect caused by reactive species in biological systems through the capture and inactivation of these reactive and potentially damaging substances. Polyphenols, constitute of group of natural products, represent a secondary metabolite of plants and that have a great variability of chemical structures. One of the fundamental structural element is the presence of hydroxyl groups which can be found in free state or bound to other forms such as; ether, ester or heteroside. The polyphenols are abundant in vegetables, and are important part of human and animal nutrition. We can find polyphenols in large quantity in fruits an Flavonoids flavonoids are further divided into many subclasses: anthocyanins, flavan- 3-ols (as monomers forming catechins and as polymers forming proanthocyanidins (condensed tannins), flavones, flavanones, flavonols, isoflavones, neoflavonoids and chalcones. They consist of a C6-C3-C6 backbone. Anthocyanidins have a basic structure, known as flavyliumcation, which contains positive charge in the C-ring, and by this positive charge it is distinguished from other flavonoids. In nature, highly unstable anthocyanidins are protected from degradation by glycosylation (anthocyanidins with sugar group(s) are called anthocyanins) generally with a number of sugars, in particular glucose, sophorose, rut nose, galactose, arabinose and xylose at different positions of the C-ring. Anthocyanins are water soluble pigments, providing the distinctive and vibrant palette of colors (red, blue and purple) found in fruits, vegetables, flowers, and other plant tissues or products. Anthocyanins are widely distributed in human diet including certain varieties of cereals and certain vegetables (cabbage, beans, onions and radishes), there are six main anthocyanidins distributed throughout the plant kingdom: cyanidin, malvidin, delphinidin, peonidin, petunidin and pelargonidin. Among them cyanidin is the most common anthocyanidin in food. Food content Phenolic acids are further divided into benzoic acid and cinnamic acid and consisting of a C1-C6 and C3-C6 backbones, respectively. They contain protocatechuic acid, vanillic acid, gallic acid and syringic acid as examples of phenolic acids in the former; and pcoumaric acid, caffeic acid, chlorogenic acid, cryptochlorogenic acid, neochlorogenicacid, ferulic acid and sinapic acid in the latter.29 Stilbenes are diphenylethene barely present in human diet, a main member is resveratrol. Lignans consists of two phenylpropane units. This group of polyphenol is further metabolized by the intestinal microflora into enterodiol and enterolactone. In general the polyphenols are well known to protect the agents a variety of diseases actions, including their ability to improve the lipid profile; dilate blood vessels by stimulating the endothelial formation of NO, and also in some blood vessels EDHF, and by their antioxidant properties, which will prevent the degradation of NO by superoxide anions (Ndiaye *et al*., 2003; Frankel et *al.*, 1993; Madeira *et al.*, 2009) .In addition, polyphenol-rich sources plants extract have been shown to reduce systolic blood pressure and to improve endothelial dysfunction in several experimental models of hypertension

(Maderia *et al*., 2009; Lee *et al*., 2011)

.Oxidative stress is a major event occurring during biological and pathological processes. Molecular oxygen (O2) is fundamental for the surviving of all aerobic organisms. Aerobic energy metabolism relies on

oxidative phosphorylation in mitochondria. However, during this process, partially reduced and highly reactive O2 metabolites, such as superoxide anions (O2-), hydrogen peroxide (H2O2) and hydroxyl radical (•OH), can be found within the cells. These chemically reactive molecules containing oxygen are called reactive oxygen species. The generation of reactive oxygen species in a biological environment exposes most living organisms to the so called 'oxygen paradox': oxygen is necessary for life and reactive oxygen species have

important roles in redox homeostasis and cell signaling but they are also potentially hazardous since reactive oxygen species may easily become a source of cell stress and tissue injury, like in most acute and chronic inflammatory processes and infections. However, most living organisms have mechanisms and strategies to overcome the excess generation of reactive oxygen species and oxidative stress, as well as to 'make use' of reactive oxygen species under physiological conditions.

Arterial thrombosis is the formation of a blood clot, thrombus, as a result of the

activation of platelets and the coagulation cascade, obstructing the flow of blood

through the circulatory system. Activation of platelets is critical to the development of platelet-rich arterial thrombosis in part because activated platelets adhering to vascular cells generate lipid peroxides and More over previous experimental animals and humans indicate the ability of polyphenol from grape-, tea-, berry-, and plant-derived products such as tea to reduce platelets30 aggregation theses effect may also have related at least to reduced risk of CVD associated with an improvement of endothelial dysfunction involving an increased formation of NO and/or NO bioavailability. Several in vivo studies have also reported vasoprotective effects by various natural products in animal models, our results indicate that strawberry tree extract are able to reduce platelets aggregation induced by collagen. Improvement from different studies also indicate the major phenolic compounds present in the strawberry tree extract are pelargonidin- 3-O-glucoside, coumaroyl- O-glucoside, and trans-cinnamoyl- O-glucoside. previous that strawberry tree is rich in phenolic compound The major groups of phenolic compounds present anthocyanins, flavonols, flavanols, ellagitannins, Gallo tannins, proanthocyanidins, and phenolic acids . our finding also indicates that the plant extract of Arbutus pavarii.

The fact that polyphenols are abundant in our diet makes it necessary to determine the nature and distribution of different kinds of polyphenols, so that we can identify which sources and key compounds are likely to provide an optimal protection especially in the cardiovascular system. Thrombosis is the formation of a blood clot, which is known as thrombus, inside a blood vessel, obstructing the flow of blood through the circulatory system. Pathologically, thrombosis may occur if the haemostatic stimulus is unregulated, either at 31 the level of stimulatory or inhibitory pathways. The balance between stimulatory and inhibitory pathways is also well established for platelets. Platelets are activated by various endogenous factors and a platelet- rich thrombus is formed subsequently in the lumen of the injured vessel. our observed indicate that *Arbutus pavarii* extract are able to inhibit the human platelets aggregation induced by collagen, studies indicate that the consumption of polyphenol-rich foods Ginkgo biloba extract may related to reduce the risk of acute coronary disease this effect may related to the ability of polyphenols to inhibition platelet aggregation and thus it could be potentially inhibited collagen, Induced Platelet aggregation.

5 Conclusion

Our preliminary results suggest that the *Arbutus pavarii* extract, revealed the presence of Coumarin, Falvonoid, Saponins, and Tannin.

It was inactive against HELA (cervical carcinoma cell line) and are inactive against HELA cells (cervical carcinoma cell line). had significant scavenging effects on the DPPH radical. *In vitro* the extract of *Arbutus pavarii* showed an antiplatelet effect. Our observations on polyphenols in general and on *Arbutus pavarii*, provide evidences suggesting that natural products might be of interest for improving liver diseases. In accordance with that, there are numerous reports that support the important role of polyphenols in preventing cardiovascular diseases, inflammatory diseases, and cancer. In addition to the antioxidant properties of polyphenols, their ability to modulate the activity of different kinds of enzymes and cell receptors are also of interest. The fact that polyphenols are abundant in our diet makes it necessary to determine the nature and distribution of different kinds of polyphenols, so that we can identify which sources and key compounds are likely to provide the greatest protection against different diseases. Further works need to detect the exact active

molecule of polyphenols and their metabolites plus more details about their bioavailability before we can proceed to introduce them in human research.

Overall further work needs to identify the pathophysiological process of portal hypertension and its complications like hepatopulmonary syndrome. Furthermore, it will be interesting to test both VSL#3 and PRBJ to study other organs affected by portal hypertension like lungs and kidneys. Our studies support that *Arbutus pavarii* rich in polyphenols are of potential interest for future work.

Recommendations

1-*Arbutus pavarii*, provide evidences suggesting that natural products might be of interest for future work.

2- Polyphenols from plant in preventing cardiovascular diseases, inflammatory diseases.

3- Polyphenols, are able to modulate the activity of different kinds of enzymes.

4- Polyphenols are abundant in our diet makes it necessary for our health.

5- Further work needs to identify the mechanism of action using animals models.

6- Investigate vivo the antiplatelet and antithrombotic activities of *Arbutus pavarii*

and if so to elucidate the underlying molecular mechanism.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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The Rates Incidence of Urinary Tract Infection in the Community and Development Uropathogens Resistance to Antibiotics

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A B S T R A C T

Urinary tract infections (UTIs) are common bacterial infections, it considers the second diseases that are frequent in the general population. This study aimed to resolve data of bacterial isolates among 3687 UTI patients at Sabratha Teaching Hospital during the years 1999, 2014 and 2015. Including results of urine culture test, biochemical test and antimicrobial susceptibility test. Results reveal that 398 (15.4%) uropathogens were isolated in 1999, 187(22.6%) in 2014 and 61(22.4%) in 2015. There was an increase in rates of isolation of uropathogens in 2014 and 2015 compared to 1999. *Escherichia coli* was the most common isolated uropathogen represented 69.6%, 18.7% and 19.7% of organisms isolated in 1999, 2014 and 2015 respectively. Other gram-negative represented 25.6%, 65.8% and 52.4%, in 1999, 2014 and 2015 respectively. Gram-positive cocci represented 4.8%, 15.5% and 21.3%, in 1999, 2014 and 2015 respectively. Rates of infections in females were higher (58%, 67% and 65%) compared to males (42%, 33% and 35%) in the three years. In this study high resistance was found of *E. coli* and other gram-negative rods (47%, 44.1%) to ceftriaxone in 1999 year compared to 2014 and 2015 years (19.1%, 28.4%). Also found high resistance of *E. coli* and other gram-negative rods (29.8%, 28.4%) to nalidixic acid in 2014 and 2015 years compared to 1999 year (11.5%, 15.6%). Urinary tract infections UTIs are the most common infections in the community. Also, showed increased uropathogens resistance to most antibiotics. So, this leads to a significant financial burden on the health system.

1 Introduction

Urinary tract infections (UTIs) are highly common infections that can affect any region of the urinary system and are typically caused by bacteria (Motse *et al*., 2019). Urinary tract infections (UTIs) can be classified as urethritis (limited to the urethra), cystitis (infection of the bladder), pyelonephritis (infection of the kidneys), and vaginitis (infection of the vagina). (Bissong *et al*., 2017; Fosso *et al*., 2017).

UTIs may be classified as uncomplicated or complicated (Flores-Mireles *et al*., 2015).

Uncomplicated UTIs, which are often community-onset cystitis, are more common in outpatient settings (Nimri *et al*., 2017). and affect otherwise healthy people without anatomical or neurologic abnormalities of the urinary tract. Uncomplicated UTIs are more common in women of all ages, although they can also affect some groups of men, such as older men and infant boys. (O'Brien *et al*., 2017; Tambyah and Maki, 2000) On the other hand, complicated UTIs are associated with patient-level factors that compromise urodynamics or host defenses, such as indwelling or intermittent urinary catheterization, urinary obstruction (e.g., by stones) or retention,

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immunosuppression, renal failure, renal transplantation, and pregnancy. (Levison and Kaye, 2013; Lichtenberger and Hooton, 2008)

UTIs are a major public health concern today and cause more than 150 million cases of disease annually throughout the world. (Motse *et al*., 2019). UTIs are more likely to occur in women than men over all age groups (Abou Heidar *et al*., 2019) and up to 50% of women report having had at least one urinary tract infection in their lifetime (Agarwal *et al*., 2020). Rates of UTIs are higher in women compared with men. Because the anatomy of women differs from that of men, their urethra is shorter and there is relative proximity between the urethra and the anus (Walsh and Collyns, 2020). The rates of UTIs among sexually active young women have been reported to vary from 0.5 to 0.7 per person-year, while this incidence rate among young men was only 0.01 (Rowe and Juthani-Mehta, 2013). However, the prevalence of UTIs reduces during middle age but increases in older adults. In addition, several other factors such as sexual intercourse and the use of spermicides have also been shown to increase the risk of UTI in women (Walsh and Collyns, 2020).

80%–90% of UTIs are caused by uropathogenic *Escherichia coli* (UPEC) (Abraham and Miao, 2015; Ejrnaes *et al*., 2011), while 5%–10% are due to *Staphylococcus saprophyticus* (Nickel, 2008). These infections are rarely viral or fungal but can involve a much wider range of pathogens, especially *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Streptococcus*, and *Enterococcus faecalis* (Amdekar *et al*., 2011; Flores-Mireles *et al*., 2015; Mann *et al*., 2017; Saka and Okunuga, 2017; Salvatore *et al*., 2011)

Nowadays, the rise in uropathogens (UPs) antibiotics resistance leads to significant challenges in the treatment of urinary tract infections (UTIs). (Sweileh *et al*., 2018; Shaji *et al*.,2021; Silva *et al*.,2022). Indeed, many studies are conducted annually to assess the antibiotics resistance of UPs isolated from UTI patients, and the results almost always show an increase in antibiotic resistance over time. (Magyar *et al*., 2017; Sultan *et al*., 2015; Sweileh *et al*., 2018; Shaji *et al*.,2021; Silva *et al*.,2022).

This study aims to find out the rates of urinary tract infections in community, as well as their incidence in females compared to males. In addition to knowing the types of bacteria that cause urinary tract infections and their resistance to antibiotics.

2 Materials and Methods

This study collected data of bacterial isolates among 3687 patients with urinary tract infections in the microbiology department of Sabratha Teaching Hospital during the years 1999, 2014 and 2015. Including results of urine culture test, biochemical test and antimicrobial susceptibility test. Unfortunately, there is not enough information in recent years (after 2015) to be studied due to the lack of capabilities in the hospital in recent years.

In this study, the statistical analysis was done using the Chi-squares test by the program Epi 2000 Centers for Disease Control and Prevention [CDC], Georgia, Atlanta to analyze the results.

3 Results

This study included 3687 patients with urinary tract infections who were enrolled in the microbiology department`s records of Sabratha Teaching Hospital during the years 1999, 2014 and 2015

Uropathogens isolated from patients with urinary tract infections in 2014 (22.6%) were more than uropathogens isolated from patients with urinary tract infections in 1999 (15.4%), with significant differences statistically (P<0.000002, Odds ratio: 1.60). Also, uropathogens isolated from patients with urinary tract infections in 2015 (22.4%) were more than from uropathogens isolated from patients with urinary tract infections in 1999 (15.4%), with significant differences statistically (P<0.003, Odds ratio: 1.58). On the other hand, there were no significant differences statistically in uropathogens isolated in 2014 compared to the 2015 year (P>0.05). Table (1).

Found an increase in rates of isolation of uropathogens, the rates of isolated uropathogens were higher in 2014 and 2015 compared to 1999.

Table (1): The ratio of isolated uropathogens in patients with UTIs in 1999, 2014 and 2015 years

years	Number of samples	Number of uropathogens	%
1999	2579	398	15.4
2014	827	187	22.6
2015	272	*61	22.4
Total number	3678	646	17.6

* The results include four candida species

Rates of infections in females were higher than in males in the three years represented 58%, 67% and 65% of organisms isolated from females in 1999, 2014 and 2015 respectively, while ratios of organisms isolated from males were 42%, 33% and 35% in 1999, 2014 and 2015 respectively. (Table 2).

Table (2): The ratio of patients with UTIs according to gender in 1999, 2014 and 2015 years

years	Number of samples	males		females	
		Number of samples	%	Number of samples	%
1999	2579	1082	42	1497	58
2014	827	272	33	555	67
2015	272	94	35	178	65

Escherichia coli represented (11%) in 1999 year followed by *Klebsiella spp*. (3%). While the other uropathogens represented lower percentages. Table (3).

Table (3): Isolation rate of uropathogens in patients with UTIs in 1999 year

Type of bacteria	Number of samples =2579	
	Isolated number	%
Escherichia coli	277	11
Klebsiella spp.	81	3
Enterobacter spp.	3	0.1
Proteus spp.	4	0.1
Pseudomonas spp.	14	0.5
Staphylococcus aureus	9	0.3
Enterococcus spp.	10	0.4
total	398	15.4

In the 1999 year, rates of infections in females (17.3%) were higher than in males (12.8%) with significant differences statistically (P <0.002, odds ratio=1.4.2). As well as, *E. coli* isolated from females (12.3%) was more than from males (8.5%) with significant differences statistically (P <0.002, odds ratio=1.52).

In addition, *Klebsiella spp*., *Staphylococcus aureus*, and *Enterococcus spp*. isolated from females (3.5%, 0.5%, 0.5%) were higher compared to males (2.6%, 0.2%, 0.3%) without significant differences statistically (P>0.05). Table (4)

Table (4): Isolation rate of uropathogens in patients with UTIs according to gender in the1999 year

Type of bacteria	Males Number of samples=1082		Females Number of samples=1497	
	Isolated number	**%**	Isolated number	%
Escherichia coli	92	8.5	185	12.3
Klebsiella spp.	29	2.6	52	3.5
Enterobacter spp.	3	0.3	0	0.0
Proteus spp.	2	0.2	2	0.1
Pseudomonas spp.	8	0.7	6	0.4
Staphylococcus aureus	2	0.2	7	0.5
Enterococcus spp.	3	0.3	7	0.5
Total	139	12.8	259	17.3

In the 2014 year, *Escherichia coli* represented (4.2%) of uropathogens isolated from patients with UTIs while ratios of gram-negative rods, *Streptococcus* spp. and *Staphylococcus* spp. were 14.9%, 3%, 0.5% respectively. Table (5).

Table (5): Isolation rate of uropathogens in patients with UTIs in 2014 year

Type of bacteria	Number of samples = 827	
	Isolated number	%
Escherichia coli	35	4.2
Gram-negative rods	123	14.9
Streptococcus spp.	25	3.0
Staphylococcus spp.	4	0.5
Total	187	22.6

Rates of infections in females (25.4%) were higher than in males (17%) with significant differences statistically (P<0.007, odds ratio=1.67) in the 2014 year. Also, *Streptococcus* spp. isolated from females (4.2%) was more than from males (0.7%) with significant differences statistically (P<0.008, odds ration=5.84).

The isolation rate of *Escherichia coli*, Gram-negative rods and *Staphylococcus* spp. from females (5%, 15.7%, 0.5%) were greater than from males (2.7%, 13.2%, 0.4%) without significant differences statistically (P>0.05). Table (6).

Table (6): Isolation rate of uropathogens in patients with UTIs according to gender in the 2014 year

Type of bacteria	Males Number of samples= 272		Females Number of samples= 555	
	Isolated number	%	Isolated number	%
Escherichia coli	7	2.7	28	5.0
Gram-negative rods	36	13.2	87	15.7
Streptococcus spp.	2	0.7	23	4.2
Staphylococcus spp.	1	0.4	3	0.5
total	46	17	141	25.4

Escherichia coli isolated from patients with UTIs was (4.4%) among isolated uropathogens in the 2015 year. While Gram-negative rods, *Streptococcus* spp., *Staphylococcus* spp. and *Candida* spp. represent 11.7%, 4.4%, 0.4%, 1.5% respectively. Table (7)

Table (7): Isolation rate of uropathogens in patients with UTIs in 2015 year

Type of bacteria or fungi	Number of samples= 272	
	Isolated number	%
Escherichia coli	12	4.4
Gram negative rods	32	11.7
Streptococcus spp.	12	4.4
Staphylococcus spp.	1	0.4
Candida spp.	4	1.5
total	61	22.4

The rates of isolated uropathogenes from females (28%) were higher compared to males (11.7%) in 2015 year with significant differences statistically (P<0.003, odds ratio=2.95). In addition, *Escherichia coli* was isolated from females (6.7%) and not from males (0.0%) and the difference in the isolation rates between females and males was statistically significant (P<0.02, odds ratio=undefined).

Also found the isolation rate of Gram-negative rods, *Streptococcus* spp. and *Candida* spp. higher in females (13.5%, 5.6%, 2.2%) compared to males (8.5%, 2.1%, 0.0%) without significant differences statistically (P>0.05). Table (8).

Table (8): Isolation rate of uropathogens in patients with UTIs according to gender in the 2015 year

Type of bacteria or fungi	Males Number of samples= 94		Females Number of samples= 178	
	Isolated number	%	Isolated number	%
Escherichia coli	0	0.0	12	6.7
Gram negative rods	8	8.5	24	13.5
Streptococcus spp.	2	2.1	10	5.6
Staphylococcus spp.	1	1.1	0	0.0
Candida spp.	0	0.0	4	2.2
total	11	11.7	50	28

This study found high resistance with significant differences statistically (P<0.0004, Odds ratio=3.73) of *E. coli* (47%) to ceftriaxone in the 1999 year compared to 2014 and 2015 years (19.1%). On the other hand, found high resistance with significant differences statistically (P<0.001, Odds ratio=3.25) of *E. coli* (29.8%) to nalidixic acid in 2014 and 2015 years compared to 1999 year (11.5%).

Regarding isolated gram-negative rods were more resistant (44.1%) to ceftriaxone in the 1999 year compared to 2014 and 2015 years (28.4%) with significant differences statistically (P<0.01, odds ratio=1.99). Also found high resistance with significant differences statistically (P<0.02, odds ratio=2.13) of Gram-negative rods (28.4%) to nalidixic acid in 2014 and 2015 years compared to 1999 year (15.6%). While there were no statistically significant differences in the antibiotic Augmentin. Table (9).

Table (9): Resistance of *E. coli* and Gram-negative rods in patients with UTIs to Augmentin, Ceftriaxone and Nalidixic acid in1999, 2014 and 2015 years.

Antibiotics	years							
	1999				2014&2015			
	Escherichia coli		Gram-negative rods		*Escherichia coli*		Gram-negative rods	
	The number 277 =	%	The number= 102	%	The number= 47	%	The number= 155	%
Augmentin	74	26.7	34	33.3	8	17	52	33.5
Ceftriaxone	130	47	45	44.1	9	19.1	44	28.4
Nalidixic acid	32	11.5	16	15.6	14	29.8	44	28.4

In three years found high resistance of *E. coli* and other gram-negative rods to most antibiotics used. Table (10, 11).

Table (10): Resistance of *E. coli* and Gram-negative rods in patients with UTIs to Ampicillin, Chloramphenicol, Co-trimethoxazole and Nitrofurantion in1999 year.

Antibiotics	*Escherichia coli*		Gram-negative rods	
	The number= 277	%	The number = 102	%
Ampicillin	149	53.7	52	51
Chloramphenicol	86	31	38	37.2
Co- trimethoxazole	110	39.7	35	34.3
Nitrofurantion	38	13.7	46	45

Table (11): Resistance of *E. coli* and Gram-negative rods in patients with UTIs to Ceftazidime, Cefuroxime, Ciprofloxacin and Gentamicin in 2014 and 2015.

Antibiotics	*Escherichia coli*		Gram-negative rods	
	The number= 47	%	The number= 155	%
Ceftazidime	3	6.4	26	16.8
Cefuroxime	11	23.4	45	29
Ciprofloxacin	10	21.3	30	19.4
Gentamicin	9	19	20	13

4 Discussion

Many studies conducted in the past showed that urinary tract infections were among the most common bacterial infections. Due to the high prevalence of urinary infections, it is projected that their identification and treatment will have a significant economic impact, resulting in substantial yearly healthcare costs. (Islam *et al*., 2022; Motse *et al*., 2019; Foxman, 2010; Zeng *et al*., 2022; Sammon *et al*., 2014; Stamm and Norrby., 2001; Flores-Mireles *et al*., 2015; Pezeshki Najafabadi *et al*., 2018; Medina and Castillo-Pino., 2019).

UTIs are more likely to occur in women than men over all age groups (Abou Heidar *et al*., 2019; Foxman, 2014; Geerlings, 2016) and up to 50% of women report having had at least one urinary tract infection in their lifetime (Agarwal *et al*., 2020)

In this study isolation rate of uropathogens in patients with urinary tract infections in 2014 (22.6%) and 2015 (22.4%) was more than from isolation rate of uropathogens in patients with urinary tract infections in 1999 (15.4%), with significant differences statistically. On the other hand, there were no significant differences statistically in uropathogens isolated in 2014 compared with the 2015 year. This indicates an increased incidence of urinary tract infections in the community, Although the number of patients who visited the hospital was lower in 2014 and 2015 years compared to 1999 year. This is due to the lack of facilities in government hospitals and the tendency of patients to private hospitals. The prevalence of urinary tract infections varies according to geographic and health conditions, as the deterioration of health and economic conditions has caused a high rate of infection and the spread of many diseases.

Zeng *et al* (2022) conducted a study in China, on UTIs burden in 204 countries and territories from 1990 to 2019. Data were obtained from the Global Health Data Exchange GBD Results Tool. The study showed an increase in the global number of individuals with UTIs from approximately 252.2 million to more than 404.6 million, an increase of approximately 152.4 million cases. the number of incident cases was significantly higher in females than males in all years from 1990 to 2019. Also, the number of deaths increased from approximately 99 000 to 237 000-an increase of approximately 130 000 deaths. The study results suggest a globally rising trend of UTI burden between 1990 and 2019.

The present study *Escherichia coli* was the most commonly isolated uropathogens in all years followed by gram negative rods and Gram-positive cocci.

Similar results were also reported in previous studies, a study conducted from June 2015 to January 2016 at Siddhi Memorial Hospital, Bhaktapur, Nepal, showed that *E. coli* was the most common pathogen (58.7%), followed by *Klebsiella pneumoniae* (22.5%). (Ganesh *et al*., 2019)

Another study was conducted in The Department of Pediatrics, Harran University Medical Faculty Hospital, Sanliurfa, Turkey from May 2015 to May 2017. *Escherichia coli* was detected in (58.9%) of the patients, *Klebsiella* (17.9%) and *Proteus* (15.8%). (Demir and Kazanasmaz., 2020)

Islam *et al* (2022) conducted a study in Dhaka, Bangladesh during 2016–2018. *E. coli* (51.6%) was the predominant causative pathogen followed by *Streptococcus spp*. (15.7%), *Klebsiella spp*. (12.1%),

Enterococcus spp. (6.4%), *Pseudomonas* spp. (4.4%), coagulase negative *Staphylococcus spp*. (2.0%), and other pathogens (7.8%).

A recent study was conducted at the Cumilla Medical College Hospital's Medicine Department in three phases (2011, 2016, 2021), In Bangladesh. *Escherichia coli* (62% in 2021, 86% in 2016 and 76% in 2011) and *Klebsiella* species (11% in 2021, 10% in 2016 and 11% in 2011) were the most frequently isolated bacteria over the study period. (Majumder *et al*., 2022).

The pathogenicity of uropathogens (UPs) is associated with the expression of several virulence factors (VFs), such as adhesion elements, toxins, capsules, flagella, serum resistance factors, and iron uptake systems (Rodriguez-Siek *et al*., 2005).

The prevalence of urinary tract infections in females is higher than in males. (Abou Heidar *et al*., 2019; Walsh and Collyns, 2020). Several predisposing factors might contribute to the higher prevalence of UTIs among women. (August and de Rosa, 2012). It is well recognized that UTI is more prevalent in female than in male, and the present study corroborates this generalization and correspond with many previous studies. Deshpande *et al*., 2011; Sharifan *et al*., 2006; Majumder *et al*., 2022; Ahmed *et al*, 2019; Islam *et al*., 2022).

The current study showed that urinary tract infections occurred in women more than men in the three years. higher incidence of UTI in women compared with men is explained by the anatomy of women because, compared to men, their urethra is shorter and there is relative proximity between the urethra and the anus. In addition, several other factors such as sexual intercourse and the use of spermicides have also been shown to increase the risk of UTI in women. Spermicides affect the vaginal microbial flora, which leads to a reduction in lactobacilli and allows the proliferation of potentially pathogenic bacteria in the genital tract (Walsh and Collyns, 2020). Furthermore, menopause can also significantly increase the risks of recurrent UTIs. Indeed, the reduction in estrogen levels can promote vaginal atrophy and lead to vaginal dryness and an increase in pH, which alters the vaginal flora and also reduces the level of lactobacilli. (Bleibel and Nguyen, 2022; Kasap *et al*, 2019).

Regarding resistance of uropathgens to antibiotics, this study found high resistance of *E. coli* and other gram-negative rods to most antibiotics used in three years. Furthermore, high resistance with significant differences statistically of *E. coli* (47%) and other gram-negative rods (44.1%) to ceftriaxone in 1999 year compared with 2014 and 2015 years (19.1% *E. coli*, 28.4% gram-negative rods). Also, found high resistance with significant differences statistically of *E. coli* (29.8%) and other gram-negative rods (28.4%) to nalidixic acid in 2014 and 2015 years compared to the 1999 year (11.5% *E. coli*, 15.6% gram-negative rods).

The reason for decrease in the resistance to ceftriaxone in the 2014 and 2015 years compared to the 1999 year and increased the resistance to nalidixic acid in 2014 and 2015 years compared to the 1999 year perhaps is deteriorating economic conditions and high prices, especially drugs. And because the price of ceftriaxone antibiotic is more expensive than nalidixic acid antibiotic. So, the tendency was to use nalidixic acid antibiotic in the treatment of urinary tract infections. This confirms the higher use of antibiotics leads to increased resistance of bacteria to antibiotics.

The result in the study similar to a study conducted in Bangladesh from June 2011 to June 2021 revealed the highest resistance (>70%) was observed against *E. coli Klebsiella spp*., and *Proteus spp*. to almost all the tested antibiotics except carbapenem. (Majumder *et al*., 2022).

Another study found drug resistance in 92% (n = 82/89) of samples, with most (80%) being resistant to at least two drugs. (Ahmed *et al*., 2019)

The incidence of UTIs caused by multidrug-resistant uropathogens has been increasing at an alarming rate worldwide. Such common infections can turn into life-threatening illnesses, especially in developing countries. (Mazzariol *et al*, 2017; Gupta *et al*, 2012).

Several studies have revealed that there is a correlation between virulence factors (VFs) and antibiotic resistance (Karam *et al*., 2018; Momtaz *et al*., 2013; Paniagua-Contreras *et al*., 2017; Shah *et al*., 2019; Tabasi *et al*., 2015). In general, in bacteria, whether they are UPs or not, several mechanisms such as changes in cell permeability and multiple efflux pumps, mutations of the antibiotic target, and horizontal transfer of resistance genes are responsible for the development of the antibiotic resistance (Mukherjee, 2019; Palma *et al*., 2020).

Effective treatment of patients with bacterial urinary tract infections is often dependent on pathogen

identification and antibiotic selection based on ongoing surveillance of the antimicrobial susceptibility pattern of urinary tract pathogens in specific regions. (Grigoryan *et al*., 2014)

5 Conclusions

Urinary tract infections are the most common infections in the community. Due to the high prevalence of infection in the community and hospital setting, urinary tract infections have imposed a significant financial burden on the health system. If UTIs are not treated can turn into life-threatening illnesses. prevalence of UTIs in females is higher than in males. And showed increased uropathogens resistance to most antibiotics used for the treatment of UTIs. This causes problems in future to treat UTIs.

Each center's identification of its uropathogens and antibiotics susceptibilities at specific intervals is important in terms of increased treatment success, prevention of unnecessary antibiotics use and prevention of antibiotics resistance. Also, should be reduce common use of antibiotics to avoiding development resistance of bacteria to antibiotics.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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The Evaluation of Tolerance of Six *Triticum aestivum* Genotypes to Salt Stress

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Soil salinity is one of the most environmental important obstacles facing the productivity and quality of wheat crops, However, the adverse effects of salinity could be mitigated by identifying salinity-tolerant genotypes. Therefore, this study was conducted with an aim of Evaluation of the tolerance of six Acsad wheat genotypes to salt stress, (Acsad 1398, Acsad 1508, and Acsad 1524) of bread wheat, and (Acsad 1595, Acsad 1671, and Acsad 1729) of durum wheat, under five levels of salt treatments (0, 50, 100,150, and 200 NaCl). The results indicate that salinity levels reveal significant ($p \leq 0.05$) differences in germination percentage, radical /plumule length, and seedling fresh weight. In addition, there were non-significant differences in the average of germination time. As noted genotype the A1508 genotype scored the highest average germination percentage reached (90.6 %), while genotype A1595 was superior to others in an average of radical /plumule length, and seedling fresh weight. Also, the result indicated that 200 mM NaCl of salinity stress was the most toxic for all wheat genotypes included in this study. finally, the results of this study categorize the wheat genotypes into tolerant genotypes include (A1508 and A1595), moderate tolerant genotypes include (A1398) and sensitive genotypes include (A1671, A1524, and A1729).

1 Introduction

In Libya, wheat (*Triticum aestivum* L.) ranks first in terms of planted area with about 165,000 ha and an annual yield of 200,000 tons (Alabasi *et al*., 2017) major staple food crop that provides fiber, proteins, vitamins, lipids, and minerals (Sabenca *et al*., 2021). Salinity is one of the most important environmental stresses, which threatens the growth and productivity of agricultural crops (Salih and Abdulrraziq, 2023), where believed that by 2050, areas affected by salinity are expected to cover about 50% of total land agriculture (Kumar *et al*., 2020). Soil salinity affects adversely seed germination, plant growth, photosynthesis, stomatal conductance, reproductive behavior, amino acid biosynthesis, and enzymatic activity, In addition, change physicochemical properties and reduces the microbial diversity of the soil (Kesh *et al*., 2022; Kumar *et al*., 2020), by ionic toxicity, osmotic stress, disruption of cellular homeostasis, nutrient mobilization reduction, and overproduction (ROS) like superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) in chloroplast and mitochondria (Riseh *et al*., 2021; Naveen *et al*., 2023). Identification of tolerant genotypes, capitalizing on adaptive traits, is considered one method that contributes to salinity stress tolerance and improved productivity (Ehtaiwesh, 2019), for example, a study conducted in Pakistan, on 125 genotypes of wheat germinated under salt stress In vitro, it was found 11 genotypes tolerant, 38 genotypes moderate tolerant, 48 genotypes moderate sensitive, and 28 genotypes sensitive (Khan *et al*., 2022), in

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Turkey, a study concluded by Oner and Kirli (2018) concluded the negative effect of salinity on germination times, seedling growth, and radicle weights and lengths of 7 different bread wheat cultivars, as confirmed results of a study in Libya, that wheat cultivars Sabha, Salambo, Makkawi, and Bushi were the most salt-tolerant out of the 12 genotypes (Ehtaiwesh and Rashed, 2019).

Therefore, the objective of this study was to evaluate salinity tolerance of six wheat genotypes.

2 Materials and Methods

2.1. Seed Selection

The laboratory study was conducted in the Department of Biology/Faculty of Education/Omar Al-Mukhtar University. Six genotypes of wheat were used. three genotypes of bread wheat (Acsad 1398, Acsad 1508, and Acsad 1524) and three genotypes of durum wheat (Acsad 1595, Acsad 1671, and Acsad 1729) were obtained from Arab center for the studies of arid zones and dry lands Acsad. Cleaned of impurities, and viability was tested by soaking in distilled water to get rid of empty seeds floating on the surface, were soaked in 1% sodium hypochloride solution for 3 minutes, and washed with distilled water (Salih *et al*., 2022).

2.2. Preparation of Used Solutions

The brine was prepared using sodium chloride salt (0, 50, 100,150, and 200 mM (NaCl) as follows:

1m Mol = molecular weight of the solute / 1000 * concentration

50 m Mol= molecular weight of the solute /1000 *50

50 m Mol=58.5 / 1000* 50

50 m Mol=2.925 g/L

Dissolve 2.925 g of NaCl salt in a 1000 ml volumetric flask and add distilled water to the mark, repeat the same previous steps for the rest of the concentrations.

2.3. Seed Germination

Normally, 10 seeds per Petri dish, were lined with two Whatman No.1 filter papers and incubated at room temperature. Each treatment was repeated three times, dishes were subjected to daily observation for 10 days and follow-up of germination in terms of addition of saline solution to the treated dishes. Distilled water was added to control as needed for each dish (Othman *et al*., 2018). The filter papers were changed once every two days to prevent salt accumulation due to evaporation, Germination was calculated by recording the number of germinated seeds in all treatments starting from the second day, which the first germination occurred, germination criterion is the appearance of radical outside seed cover at end of the experiment took final results of following qualities :

Germination percentage (PG %) = number of germinated seeds / total number of seeds cultured × 100 (Yousif *et al*., 2020).

Mean germination time (MGT) = The total number of germinated seeds per day / total number of germinated seeds at end of the experiment (Salih and Abdulrraziq, 2018).

Radical and plumule lengths (cm): The root and plumule lengths were taken using a graduated ruler, and the averages were calculated by taking 5 seedlings from each plate.

Fresh weight of seedling (g): The weight 5 of the seedlings of each dish was taken and the averages were taken.

Statistical Analysis

The study experience was designed according to the complete random design (CRD). Statistical analysis was performed using Minitab 17 program and ANOVA variance analysis tables. The averages were compared using Tukey's test at $P < 0.05$.

3 Results

3.1. Effects of Salinity on Germination Percentage and Mean Germination Time

The results presented in Table (1) and (2), Fig (1) and (2), represented the effect of salinity levels (0, 50, 100, 150, and 200 mM NaCl) on germination percentage and mean germination time after 10 days of germination. The recorded data showed significant differences in a decrease in germination percentage of six wheat genotypes. Where the lowest averages percentage of germination was recorded for genotypes Acsad1729, Acsad1524, and Acsad1671 from 100% for control to (61.2, 62.6, and 67.2%), and with an increase in means germination time from 3.5 days for control to (4.3, 3.9 and 4.2days) respectively. Also, caused a high concentration (200 mM) suppressed all seed growth for all wheat genotypes included in this study. The results showed that the percent germination of the genotypes (Acsad1595 and Acsad1398) was not affected at 50 and

100 mM., but decreased by (90 and 65%) at 150 mM, and (40 and 20%) at 200 mM, compared to the control of these two genotypes respectively, also this level of salinity stress caused a delay in an average germination time from (3.4 days) and (3.2 days) for control to (4.0 days) and (4.2 days) for these two genotypes respectively. The results also showed that in genotype Acsad1508 the germination percentage was not affected at 50, 100, and 150 mM, but decreased by (53%) at 200 mM, also the same level of salinity caused a delay in an average germination time from (3.2days) for control to (3.7 days).

Table (1): The effect of salinity levels on germination percentage (%) of six wheat genotypes.

Genotypes	NaCl (mM)					Genotypes average
	Control	50	100	150	200	
A1398	100	100	100	65	20	77.0 bc
A1508	100	100	100	100	53	90.6 a
A1524	100	100	80	33	0.0	62.6 d
A1595	100	100	100	90	40	86.0 ab
A1671	100	100	90	46	0.0	67.2 cd
A1729	100	100	80	26	0.0	61.2 d

Table (2): The effect of salinity levels on mean germination time of six wheat genotypes (day).

Genotypes	NaCl (mM)					Genotypes average
	Control	50	100	150	200	
A1398	3.2	4.0	4.2	4.7	5.2	4.2 a
A1508	3.2	3.5	3.8	4.3	4.6	3.7 a
A1524	3.5	4.5	5.4	6.5	0.0	3.9 a
A1595	3.4	3.7	4.0	4.5	4.8	4.0 a
A1671	3.5	5.1	5.8	6.7	0.0	4.2 a
A1729	3.5	5.2	6.3	6.9	0.0	4.3 a

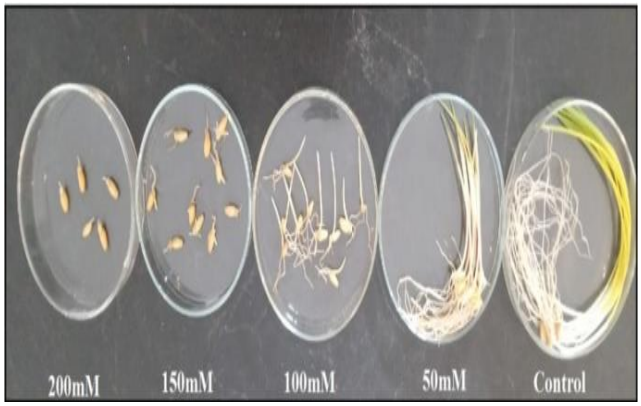

Figure (1): The effect of salinity levels on germination rates of genotype Acsad 1508.

Figure (2): effect of salinity levels on germination rates of genotype Acsad1595.

3.2. Effects of salinity on shoot and plumule length

The results presented in Tables (3 and 4) of six wheat genotypes showed significant differences in shoot and plumule length. Where the lowest average of radical length was recorded at (2.3, and 2,7cm), for genotypes (Acsad1524, and Acsad1729) respectively. However, for the rest of genotypes the radical lengths ranging between (3.5, and 5.6cm), Acsad1595 was the best among them.

Table (3): The effect of salinity levels on radical length (cm) of six wheat genotypes.

Genotypes	NaCl (mM)					Genotypes average
	Control	50	100	150	200	
A1398	9.2	6.5	1.3	0.4	0.2	3.5 cd
A1508	11.5	9.3	2.0	0.7	0.2	4.7 b
A1524	7.5	5.6	1.0	0.4	0.0	2.3 e
A1595	12.6	10.0	4.2	1.2	0.3	5.6 a
A1671	9.4	6.8	2.2	0.6	0.0	3.8 c
A1729	8.2	4.5	1.0	0.2	0.0	2.7 de

As noted, the lowest average plumule length (3.8, 4.0, and 4.2cm) for genotypes (Acsad1729, Acsad1671, and Acsad1524), respectively, and the value increased to (5, 6, and 7), for Acsad1398, Acsad1508, and Acsad1595 respectively.

Table (4): The effect of salinity levels on Plumule length (cm) of six wheat genotypes.

Genotypes	NaCl (mM)					Genotypes average
	Control	50	100	150	200	
A1398	11.7	10.0	2.6	0.5	0.3	5.0 ab
A1508	13.4	11.3	3.7	1.2	0.4	6.0 ab
A1524	10.5	7.5	3.0	0.4	0.0	4.2 b
A1595	15.0	11.2	6.9	1.6	0.3	7.0 a
A1671	9.5	7.2	3.1	0.2	0.0	4.0 b
A1729	9.9	8.0	1.2	0.3	0.0	3.8 b

3.3. Effects of Salinity on Seedling Fresh Weight

The results in Table (5), of six wheat genotypes, showed significant differences in average seedling fresh weight. Where the highest average of seedling fresh weight was recorded (1.43g), for A1595, followed by A1508 (1.30 g), while the remaining genotypes had an average seedling fresh weight ranging between (0.91 and 1.14 g), A1729 and A1671 were the lowest genotypes.

Table (5): The effect of salinity levels on seedling fresh weight (g) of six wheat genotypes.

Genotypes	NaCl (mM)					Genotypes average
	Control	50	100	150	200	
A1398	2.50	1.65	0.90	0.54	0.13	1.14 c
A1508	2.87	1.80	1.04	0.86	0.15	1.30 b
A1524	2.33	1.71	0.82	0.40	0.0	1.05 cd
A1595	2.94	2.10	1.09	0.73	0.32	1.43 a
A1671	2.41	1.32	0.78	0.35	0.0	0.97 de
A1729	2.43	1.20	0.80	0.12	0.0	0.91 e

4 Discussion

Wheat (*Triticum aestivum* L.) occupies nearly 200 million hectares with a global production of approximately 716 million tons, the second most important cereal crop in the world (Mamrutha *et al.*, 2019). However, wheat crops suffer physiological and biochemical changes when exposed to salt stress, which leads to a decrease in yield and productivity (Masarmi *et al.*, 2023; Khalid *et al*., 2023). Therefore, this study was conducted on the verification of tolerance of six Acsad wheat genotypes to salt stress. Which showed highly significant differences when ($P < 0.05$) in reducing germination percentage, lengths of radical and shoot, and decreasing seedling fresh weight in wheat genotypes included in this study compared to control, by salinity stress. Different germination rates were reported for genotypes of wheat for salinity tolerance (Uzair *et al*., 2022; Quan *et al*.,2021). May be attributed to osmotic and ion-specific toxic effects, which reduce the amounts of seed germination stimulants such as GAs, enhancing ABA amounts, and reducing seed absorption of water, also Na+, can replace the K+ cation and promote ion toxicity, which changes the permeability of the membranes and damage (Assaha *et al*., 2017; Adhikari *et al*.,2022). Moreover, the A1508 genotype scored the highest average germination percentage reached (90.6 %), while genotype A1595 was superior to others in average Radical /Plumule length, and seedling fresh weight. Also, the study found that concentration of 200 mM NaCl is the most toxic to all genotypes. Our results categorize wheat genotypes included in this study into tolerant (A1508 and A1595), moderate tolerance (A1398), and sensitive (A1671, A1524, and A1729). The different behavior of genotypes in salinity tolerance could be attributed to genetic variation (Munns and Tester, 2008; Al-Ashkar *et al*., 2020).

5 Conclusion

In this study, Six genotypes from the Arab Center for the Studies of arid and dry lands (Acsad) were investigated for their tolerance to different levels of salinity. Salinity reduced all the studied traits of the genotypes. The study categorizes the wheat genotypes into tolerant (A1508 and A1595), moderate tolerance (A1398) and sensitive (A1671, A1524 and A1729). The authors concluded that salt tolerance depends on their wheat genotype.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Proteus Genus Sensitivity Testing for Various Classes of Antibiotics

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Proteus genus has become one of the most common pathogens in Libya, with high antibiotic resistance, which can lead to medical problems in many situations in hospitals. However, there is no comprehensive study of the sensitivity and resistance of Proteus pathogenic to antibiotics in Libya. Therefore, the present study was conducted with the aim to test the sensitivity and resistance of two *Proteus* isolates (UTI and diarrhea) to antibiotics. The Kirby-Bauer (disc diffusion) method was used to investigate the effects of eight Antibiotics, belonging to different classes. Aminoglycosides represented by Gentamicin, Tetracyclins class represented by Doxycycline 30ug, Penicillin class represented by Ampicillin 10ug, Macrolides class represented by (Azithromycin 15ug, Erythromycin 15ug), and Cephalosporins class represented by (Ceftriaxone 30ug, Ceftazidime 30ug, and Cephalexin 30ug). Antibiotic susceptibility results test revealed all proteus isolates to be resistant to most antibiotics, especially the classes (penicillin, Macrolides, Cephalosporins). In contrast, there were no significant differences between the resistance of the protease isolates from the urinary tract and the protease isolated from diarrhea. On the other hand, the antibiotic gentamicin recorded the highest sensitivity to *Proteus* isolates (UTI) and (diarrhea) tested at 46.6% and 39.9%, respectively. This study concludes to new antibiotics must be developed, although aminoglycosides are still effective against *Proteus* genus.

1 Introduction

Libyan society is among the societies using practice self-medication with antibiotics without a physician's prescription, which has led to bacterial resistance to these antibiotics and the emergence of pathological cases, largely unresponsive to drugs (Meerah, 2023; Hosien *et al*., 2022). Since the discovery of antibiotics, Drug manufacturers rely on small molecules derived from microbes, as well improvements in traditional antibiotics have led to the development of various new antibiotics (Shim, 2023), but with the increased antimicrobial resistance, new generations of antibiotics had to be developed more efficiently (Frieri *et al*., 2017). Enterobacteriaceae are important pathogens in nosocomial and community settings, in the last years, antimicrobial resistance in this family has increased dramatically worldwide (Rozwandowicz *et al*., 2018).

Proteus ssp is a gram-negative bacterium, facultatively anaerobic and heterotrophic, usually rod-shaped. It shows a change in shape, is mobile, urease-positive, belongs to Enterobacteriaceae, and is responsible for urinary tract infections. Gustav hauser first described it in 1885 and it is characterized by intensive swarm growth on solid ground. (Mohsin and AL-Rubaii, 2023; Drzewiecka, 2016). These bacteria are found in wastewater and soil, although considered commensals in the digestive system, they also possess many

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virulence factors that may be relevant to gastrointestinal pathogenicity (Hamilton *et al*., 2018). At first, the genus was divided into two species based on the liquefying gelatin. Lately, new seven species have been detected, the genus *Proteus* thus includes ten species and three unnamed genotypes (Hyun *et al*., 2016; Dai *et al*., 2020), among them were *P.vulgaris* and *P.mirabilis*, the most related causative agents of urinary tract infections (Rozalski *et al*., 1997). Also, they cause various infections, such as wound infections, diarrhea and meningitis in newborns (Phan and Lehman, 2012). High resistance to *Proteus* spp has been reported frequently, by their ability its ability to form crystalline biofilms, in addition, to moving, transferring, adhere, the release of endotoxins and enzymes such as urease, hemolysin, protease and DNase (AL-Dulaimy *et al*., 2023; Jalil *et al*., 2023). Moreover, It was confirmed that this genus has carbapenemase genes that serve to break down the antibiotic and reduce its effectiveness (Al-Nabhani and Shami, 2023), in addition to producing plasmids (Hua *et al*., 2020). In contrast, a recent study found that the synergistic effect of antibiotics could have an antagonistic effect against the growth of these bacteria (Mohsin and AL-Rubaii, 2023). It was found that both the blaTEM and *qnr* genes from *Proteus* spp. involved in conferring resistance to β-lactam and quinolone antibiotics (Bilal *et al*., 2019). However, there is no complete study of the resistance and sensitivity to *Proteus* genus against antibiotics in Libya.

The aim of this study was therefore to provide information on the effectiveness of common antibiotics in combating *Proteus* genus isolates in Libya.

2 Materials and Methods

2.1. Bacterial Isolates

The isolates of *Proteus* spp identified were obtained from an Al-Mejhar laboratory, Al-Bayda City, based on characteristic growth on blood agar, and non-lactose-fermenting colonies on MacConkey's agar media, Fig(1).

- *Proteus* (UTI): isolate from urine tract infection.

- *Proteus* (D): isolate from diarrhea.

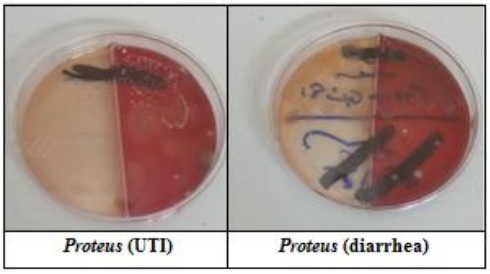

Figure (1): *Proteus* isolates on MacConkey's and blood agar media.

2.2 Antimicrobial Susceptibility Test

The media were sterilized in an autoclave at 121 °C for 15 minutes, and the bacteria were grown on Mueller—Hinton Agar. A routine antimicrobial susceptibility test was performed by the Kirby–Bauer disk diffusion method against *Proteus* spp. For screening, a disc antibiotic was placed on the surface of the inoculated bacterial medium. The dishes were incubated at 37°C for 20–24 hours in triplicate per dish. The zone of inhibition diameter minus the disc diameter was measured. This study used 8 types of commonly used antibiotics including: Doxycycline (DO) 30µg, Gentamicin (CN) 10µg, Ampicillin (AMP) 10µg, Azithromycin (AZM) 15µg, Cephalexin (CL) 30µg, Ceftriaxone (CTR) 30µg, Ceftazidime (CAZ) 30µg, Erythromycin (ERY) 10µg (Abdulrraziq and Salih, 2022).

Statistical Analysis

The study experiences tense designed according to the complete random design (CRD). Statistical analysis was performed using Minitab 17 program and ANOVA variance analysis tables. The averages were compared using Tukey's test at P <0.05 (Abdulrraziq *et al*., 2023).

3 Results

Table (1) and Fig. (2, 3 and 4). The results represent of antibiotic susceptibility testing of Proteus spp. isolates against 8 types of commonly used antibiotics, which showed a wide variation in their antibiotic resistance.

3.1. Sensitivity of *Proteus* isolates (UTI) to antibiotics

The highest was percentage of sensitivity to the antibiotic Gentamicin at a diameter of (5.9mm) by (39.3%), followed by Doxycycline at a diameter of (4.6mm) by (30.6%), while middle-sensitivity to Erythromycin and Ceftriaxone by (20.0 and 15.0%) respectively. The isolate showed high resistance to the

rest, antibiotics, including (Ampicillin and Ceftazidime), were recorded sensitivity (6.0%), and Azithromycin by (3.0%). Finally, the highest percentage of resistance to the antibiotic Cephalexin was 100%.

Table(1): Effect of various antibiotics against *Proteus* isolate (UTI).

Class / Antibiotic / Isolate			Urine tract infection (UTI)		
			Zone inhibition (mm)	Sensitivity (%)	Resistance (%)
Tetracyclins	Doxycycline (DO)	30	4.6+0.3	30.6	69.4
Aminoglycoside	Gentamicin (CN)	10	5.9+0.5	39.3	60.7
Penicillin	Ampicillin (AMP)	10	1.0+0.0	6	94
Macrolides	Azithromycin (AZM)	15	0.5+0.0	3.3	96.7
	Erythromycin (ERY)	15	3.0+0.1	20	80
Cephalosporins	Ceftriaxone (CTR)	30	2.3+0.0	15.3	84.7
	Ceftazidime (CAZ)	30	1.0+0.0	6	94
	Cephalexin (CL)	30	0.0+0.0	0	100

3.2. Sensitivity of *Proteus* isolates (Diarrhoea) to antibiotics

Proteus isolate showed maximum sensitivity to gentamicin (46.6%), which was the highest sensitivity ratio recorded in this study, while antibiotics (Ceftriaxone, Ampicillin, Ceftazidime, Erythromycin and Doxycycline) recorded sensitivity ranged between (10-20%). On the other hand, it was found that Proteus isolate showed maximum resistance (100.0%) to two antibiotics; Cephalexin and Azithromycin.

Table(2): Effect of various antibiotics against *Proteus* isolate (diarrhea).

Class / Antibiotic / Isolate			Diarrhea		
			Zone inhibition (mm)	Sensitivity (%)	Resistance (%)
Tetracyclins	Doxycycline (DO)	30	3.0+0.3	20	80
Aminoglycoside	Gentamicin (CN)	10	7.3+1.0	46.6	53.4
Penicillin	Ampicillin (AMP)	10	1.8+0.2	12	88
Macrolides	Azithromycin (AZM)	15	0.0+0.0	0	100
	Erythromycin (ERY)	15	2.3+0.2	15.3	84.7
Cephalosporins	Ceftriaxone (CTR)	30	1.5+0.0	10	90
	Ceftazidime (CAZ)	30	2.2+0.3	14.6	85.4
	Cephalexin (CL)	30	0.0+0.0	0	100

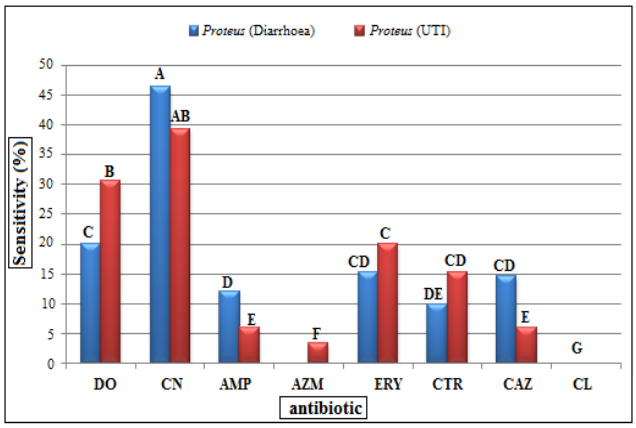

Figure (2): Sensitivity of *Proteus* isolates to antibiotics.

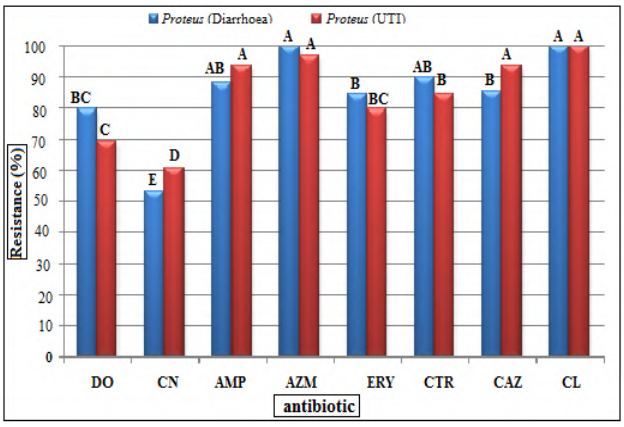

Figure (3): Resistance of *Proteus* isolates to antibiotics.

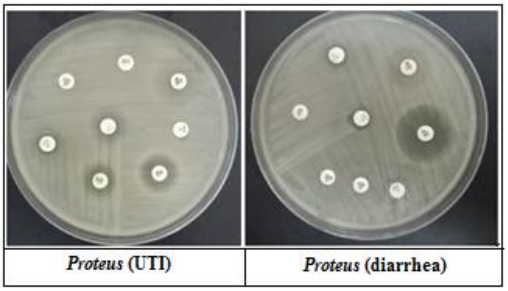

Figure (4): Effect of various antibiotics against *Proteus* isolate (UTI and diarrhea).

4 Discussion

The characterization and dissemination of antimicrobial resistance profiles provide useful details on the potential challenges in treating bacteria (Jalil *et al*., 2023). In this study, the high percentage sensitivity of *Proteus* spp isolates to Gentamicin 46.6 and 39.3%. This result is similar to the study that was reported by Flamerz *et al*. (2023). It was supported by McMurtry *et al*. (2021) who noticed that aminoglycosides effectiveness against negative gram bacteria, where this group of antibiotics binds to disrupt protein translation, leading to widespread cell damage and bacterial cell death (Webster and Shepherd, 2023). Also, the group of tetracyclins antibiotics represented by Doxycycline had reasonable activity against Proteus isolates, This result agrees with (Alqani *et al*., 2023), a study also pointed to the possibility of re-treating and advancing the three generations of the tetracyclins class in treating bacterial infections (LaPlante *et al*., 2022), by binding tetracycline particles to 16S rRNA using the ribosomal subunit of bacteria (Chukwudi, 2016), As for the penicillin represented by Ampicillin, the percentage of sensitivity of the isolates against this antibiotic in the current study was between (6-12%), This means that the resistance rate is about 90%. The penicillin class of antibiotics is known to be effective in treating both infectious and non-infectious diseases, however, biofilm formation by *Proteus* species can impede the action of these antibiotics (Li *et al*., 2022). As for the sensitivity of class antibiotics Macrolides (Erythromycin and Azithromycin), bacterial isolates resistance was high, between (80 -100%), These results agree with (ALjeelizy *et al*., 2022). Finally, the highest rates of resistance were against class Cephalosporins antibiotics represented by (Ceftriaxone, Ceftazidime, and Cephalexin) in this study. This result is consistent with a study, which reported a decrease in the clinical effect of cephalosporins series with frequent use (Shipitsyna and Osipova, 2022).

5 Conclusion

Generally, In the present study, all Proteus ssp isolates were shown to be multi-antibiotic resistant. There were no significant differences between the resistance of protease isolated from the urinary tract and those isolated from diarrhea. In addition, aminoglycosides are still effective in combating Proteus bacteria. Although, the development of generations of drugs, it should be that noted self-medication and long-term antibiotic therapy can lead to the emergence of resistant isolates.

Conflict of Interest: The authors declare that there are no conflicts of interest**.**

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Secure Key Exchange Using Boolean algebra: A New Method Based on NP-Hard Problem

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A B S T R A C T

Secure key exchange is essential for maintaining the confidentiality and integrity of transmitted data in contemporary communication systems. To restrict unwanted access to the transferred keys, traditional key exchange techniques relied on computational complexity. Traditional approaches could be attacked, though, if modern computing resources become more powerful. This article suggests a novel method for secure key exchange based on NP-hardness and Boolean algebra. The method creates a public value from the private keys of two participants and other information, and each person then uses their own private key and the other public value to obtain the shared key. The fact that the private keys are not disclosed and that both users compute the secret key using their respective sets of private keys and values received from the other side makes the system resistant to man-in-the-middle attacks. The major goal of the suggested solution is to safely retrieve the same value as a shared key for both participants, even if others already know the public values. Boolean algebra and an NP-hard issue offer higher security assurances than conventional techniques that only consider computational complexity. The study uses a key size of 128 bits, which produces good results and offers higher security guarantees than conventional techniques. The study has also created a brand-new key exchange strategy that enhances current methods. Overall, this method marks a substantial advancement in the use of Boolean algebra and NP-hard issues to achieve secure key exchange.

1 Introduction

The development and extension of the network require a system to secure the transmitted information through the network. Cryptography can offer this service by making communication secure over the network (Stallings, W. 2017). So the main goal of implementing cryptography is to keep the network information communicating through a secure channel, so the stored information may be accessed without proper authorization, but it is still secure, and the secret data cannot be obtained even if the transmitted messages are able to be read.

Most of the methods use the echo of the complexity of that method, and that complexity is used to generate a coded text or message to hide the original text or message (Paar & Pelzl,. 2010). The way to use that method to generate a coded message is called a key.

There are many kinds of keys, and each one has a different technique to implement it, and the difference depends on how to use or achieve that key. One type of key is called the public key, which has many methods to make or generate that public key (Katz & Lindell. 2014); each method depends on the way it is used and

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the properties of that method to encode the information and get secure communication over the network.

The Boolean algorithm is one of the methods used to generate a public key (Wu & Chen. 2017). The Boolean operations have properties that make it possible to get different values by implementing them.

The idea is to implement the Boolean operations on the data and get another new date; the new date is supposed to be different than the original data. That property of Boolean operations forces many people to study and implement it to generate a public key (Li, & Zhang. 2019).

Even though there are some ways to achieve key sharing, it is possible to find a new method to achieve the same goal, maybe with better performance (Diffie & Hellman. 1976). By using the Boolean properties, it is possible to make a new method to distribute the keys.

There is a problem called the NP-hard problem, and depending on that problem, it is possible to share keys in a secure way (Rivest, et. al. 1978).

This study proposes a new secure approach for key exchange using Boolean algebra based on an NP-hard problem. The proposed approach generates a shared key that is computationally difficult to determine, even if an attacker has access to the public values exchanged during the key exchange process (Singh & Babu . 2018). By leveraging the properties of Boolean algebra and an NP-hard problem, this approach provides stronger security guarantees compared to traditional methods that rely solely on computational complexity. The proposed study presents a novel approach to key exchange that improves upon existing techniques (Yizhi H, et. al, 2023). This approach represents a significant step forward in achieving secure key exchange in modern communication systems. However, this study can be used as an alternative to other key-sharing methods, such as Diffie-Hellman, in a faster way (Vetrivelan & Padmavathi, 2019).

2 Previous Studies

A technique known as cryptography is used to ensure the security and protection of data or information while it is transferred and exchanged over a network, or to make it invisible to others, possibly because the owner of the data or information does not want it to be seen or because it contains secret information. Emails, credit card numbers, websites, and a variety of other items are protected using cryptography (K. Ahmed, et. al, 2023).

Information can be concealed via cryptography, and when concealed information is communicated through a network, nobody can decipher what it implies. (Stallings, W. 2017).

By encrypting the data to "make it non visible or non-understandable" or to transform the contents of a message into a format that cannot be read," As stated by the sender, and decrypting it to "make the data understandable" or to transform the message back into a readable format," as stated by the receiver, cryptography provides this service. When a communication is encrypted, it is known as cipher text, and when it is decrypted, it is known as plaintext (Paa & Pelzl . 2010).

The original text communication is known as the plaintext. Cipher text is the encrypted message created by employing the algorithm and secret key on the plaintext message. (Diffie & Hellman.1976).

There are many different types of keys that can be used for both encryption and decryption. A variety of cryptography systems are categorized. Depending on how the key(s) are used.

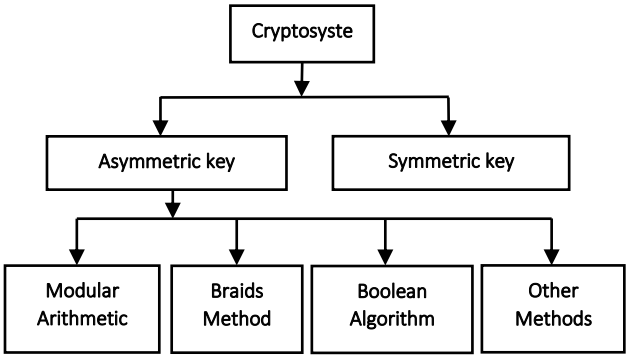

Figure (1). Types of keys

Certainly! The study of cryptography focuses on the methods and formulas used to protect data transfer and communication. The purpose of cryptography is to protect data's secrecy, integrity, and authenticity while it is being stored in a system or transferred over a network (Paar, et. al, 2010).

The original message, known as plaintext, is converted into an unintelligible format, known as cipher text, using a variety of encryption techniques. Symmetric-key encryption and public-key encryption are the two

encryption methods that are most frequently employed (Katz & Lindell. 2014)

The same secret key is employed in symmetric-key encryption for both encryption and decryption. This implies that secure communication requires a shared key between the sender and the recipient. Advanced Encryption Standard (AES) and Data Encryption Standard (DES) are the two most widely used symmetric-key encryption methods (Schneier, B. 1996).

Two separate keys are used for encryption and decryption in public-key encryption, sometimes referred to as asymmetric encryption. The private key is used to decode data, whereas the public key is used to encrypt data. RSA is the most widely used public-key encryption algorithm (Menezes, et. Al. 2010)

Numerous applications, such as secure online transactions, email encryption, secure messaging services, and secure communication networks, utilise cryptography. In order to guarantee the integrity and authenticity of digital documents, it is also utilized in digital signatures (Ferguson, et. al, 2010)

Overall, cryptography is essential for protecting communication and data transmission in the current digital era, and it is ever-evolving as new dangers and weaknesses are discovered.

2.1. Encryption and Decryption

2.1.1 Symmetric key

The sender encrypts the information using a certain key, and the receiver decrypts the encrypted information using the same key in a symmetric key system. Both "sender" and "receiver" must be aware of this key.

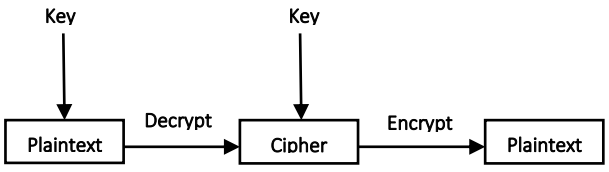

Figure (2). Symmetric key

Let's say sender A wishes to send a message to receiver B. Sender A would use a key to encrypt the message and convert it to cipher text before sending it to recipient B via the network. This message is delivered as a cipher text to the receiver B side.

To obtain the original message or plain text, recipient B should decrypt it using the same key that the sender used, (Buchmann, J. 2001).

2.1.2 Public Key or Asymmetric Key

This it uses two keys, a public key and a private key. Data encryption using public key cryptography is effective. Anyone sending a message can use the recipient's public key to encrypt the message and make the recipient's public key available. If a recipient has a private key, can share it with other people so can send those messages. Everyone should be aware of this key in order to be able to send messages to the owner of the public key by using it to encrypt the message and convert it to cipher text. Only the owner of the public key can then decrypt the message. The issue with public key systems is that in order to encrypt a message using the recipient's public key. As shown in the figure (3), (Schneier, B. 1996).

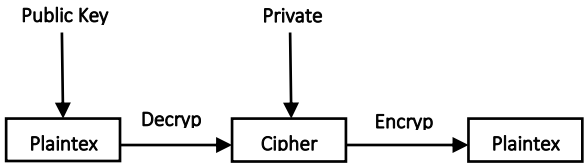

Figure (3). Public key or asymmetric key

Only the owner of the private key may determine the meaning of the message that has been given, making the private key a secret key. To recover the original communication's "plaintext," the owner of this key can decrypt a message that has been encrypted with a public key (Mao, W. 2003).

Sender A wants to communicate with recipient B. By utilizing the recipient's public key, A should encrypt the message and convert it to cipher text. Then deliver it through the network to receiver B. This message is delivered as a cipher text to the receiving side. In order to decrypt it and obtain the message's plaintext, recipient B should use his or her owner private key (Menezes, et. al, 1996).

It is impossible to know the private key if you know the public key since the public key can only be used to encrypt messages and only the associated private key can be used to decrypt those messages.

2.2 The Distribution of the Keys

The distribution of keys is one of the encryption's key functions. The use of public key cryptography has two components. The secret keys are distributed via the public key distribution, not the other way around. Numerous methods have been suggested for the keys distribution in order to accomplish this.

A. The Secret Key Exchange

There are numerous issues with sharing and exchanging secret keys, which is one of the two causes for the invention of public key cryptography.

To help us examine conventional methods of distributing secret keys as well as usual issues with secret key distribution, let's reintroduce Alice and Bob and their children, Casey and Dawn. Currently retired, Alice and Bob are residing in the upper Midwest. Casey, their son, has taken over the California Company. (Stallings, W. 2013)

The year DES became a standard was 1977. To describe common issues with secret key distribution as well as the conventional methods of doing so. Let's say Alice and Bob need to communicate each other some info. In this situation, Alice and Bob must create their own DES secret key. Bob communicates with Alice via DES.

Casey wants to send something to Bob on behalf of another individual. Bob is asked for his private key as a result. Bob will receive the messages between them if he uses the same DES that he did with Alice. To provide Casey, Bob creates another DES secret key (Ferguson, et. al, 2015).

B. The Problem and the Traditional Solution

How are Casey and Alice able to give him the secret key? In other words, how can it be sent to him? They are unable to guarantee the secret key's security during transportation; hence they are unable to send it through mail. The secret key might be given to Casey by Alice and Bob in person or by a reliable courier. This approach is outdated. If only the three users Casey, Bob, and Alice know the secret key. So that they can pass messages back and forth (Bellare, et. al, 2015)

What if Dawn also requests a private key? She requests that Alice and Bob create a new secret key for her because she does not want to use the one that the others share with Casey. Therefore, Alice (or Bob) must give Dawn a secret key.

The three secret keys that Alice and Bob now possess are one that they use exclusively, one that they share with Casey, and one that they also share with Dawn (Paar, et. al, 2016).

2.3 Boolean Algorithm

George Boole (1815–1864) created the Boolean logic, which is typically used to fine-tune the determination of system status or to set or clear certain bits. A technique to compare individual bits is Boolean logic. It can choose the 'operators' to specify how the bits are compared and the outcome. Boxes with several inputs and a single output are preferred by operators. The result will either be 0 or 1.The operations that are used the most frequently are AND operation", OR operation", and NOT operation" (Boneh, & Shoup, 2017).

The main concept is to incorporate Boolean operation expressions into the cryptosystem for encryption and decryption. The security of this algorithm depends on how challenging it is to find a task that satisfies the provided set of Boolean expressions (Li, & Zhang, 2020). This algorithm also involves security concerns and the use of public key cryptosystems.

The Boolean permutation is used in this work to create the public key cryptosystem. The Boolean permutation's inversion forms the foundation of this method's security (Singh, & Babu. 2021). The Boolean permutations have a variety of characteristics that can be employed in cryptography to ensure security.

If the following conditions are met, the Boolean permutation can be used to generate the public key:
With some expertise, finding the inverse of the Boolean permutation is simple (Katz, & Lindell. 2014).

It is computationally impossible to obtain the inverse of the permutation without prior knowledge.

The trapdoor is a special piece of information that must be understood in order to determine the inverse; one method for creating a Boolean permutation entails using a composition of Boolean permutations.

3 Materials and Methods

3.1 NP-Problem

A problem is called a NP (nondeterministic polynomial time) class if it is can be solved in polynomial time by a nondeterministic Turing machine.

The NP-complete problems are the most difficult problems in NP, that is because if one could find such way to solve any NP-complete problem quickly (in polynomial time), then it will be possible to use that algorithm to solve all NP problems quickly (Goldreich, O. 2017).

One example of an NP-complete problem is the Boolean satisfiability problem.

3.2 Boolean Satisfiability Problem

The Boolean satisfiability problem (SAT) is a decision problem considered in complexity theory. A formula of the problem is a Boolean expression written using only AND, OR, NOT, variables. The purpose was given the expression, is there some assignment of TRUE and FALSE values to the variables that will make the entire expression true (Zeng, & Yu. 2020)

Mathematically, a formula of propositional logic is said to be satisfiable if logical values can be assigned to its variables in a way that makes the formula true. The class of satisfiable propositional formulas is NP-complete (Boneh, & Shoup. 2015).

The problem can be significantly restricted while still remaining NP-complete.
By applying De Morgan's laws, assume that NOT operators are only applied directly to variables, not expressions; we refer to either a variable or its negation as a literal. For example, both x1 and not(x2) are literals, the first a positive literal and the second a negative literal. Together a group of literals, get a clause, such as (x1 or not(x2)). Finally, let us consider formulas that are a conjunction (AND) of clauses. This called conjunctive normal form. Determining whether a formula in this form is satisfiable is still NP-complete, even if each clause is limited to at most three literals. This last problem is called 3SAT, 3CNFSAT, or 3-satisfiability (Katz, & Lindell. 2019).

On the other hand, restrict each clause to at most two literals, the resulting problem, and 2SAT, is NL-complete. Alternately, if every clause must be a Horn clause, containing at most one positive literal, the resulting problem, Horn-satisfiability, is P-complete,

3.2.1 Complexity

SAT is considered a NP-complete. As proved by Stephen Cook in, the issue of an NP-complete problem did not even exist (Rogaway, P. 2015). The problem remains NP-complete even if all expressions are written in conjunctive normal form with 3 variables per clause (3-CNF), yielding the 3SAT problem. In this case the expression has the form:

(x11 OR x12 OR x13) AND (x21 OR x22 OR x23) AND (x31 OR x32 OR x33) AND...

When each x is a variable and each variable can appear many times in the expression.
A good useful property is that it preserves the number of accepting answers.

3.2.2 Satisfiability

It is a special case of k-satisfiability (k-SAT). When each clause contains at 3 variables.

E = (x1 or ~x2 or ~x3) and (x1 or x2 or x4) and (x1 or x3 or~ x4)
E has three clauses, four literals (x1, x2, x3, x4), and k=3 (three variables per clause).

Here to get a solution to this equation of the decision problem must determine whether there is a truth values (TRUE or FALSE) that can assign to the variables (x1 through x4) such that the entire expression is TRUE. For example, the next values to the x's variables (x1 = TRUE, x2 = TRUE, x3=TRUE, x4=TRUE), so the answer in this case is YES. This is one of many possible assignments (Canetti, R. 2016). Any set includes at least one TRUE value, it is enough to get YES answer. If there were no such assignment(s), the answer would be NO.

3.3 Key Sharing Using Boolean Satisfiability Problem

Depending on the Boolean satisfiability problem NP hard problem proprieties, and the main issue of the key exchanging, it is possible to achieve new way for key exchange the keys using the Boolean satisfiability problem, In this case it is possible to use 3- satisfiability to achieve key exchanging, Because of the properties

'as it shown prior ', so it allows to exchange the values is secret way.

Let to assume Alice and Bob attempt to exchange a secret key (Boneh, & Shoup. 2019). First of all, both of the users have to choose an integer random privet value. Alice chooses a, and Bob chooses b. and both of them agree on the same public value z. Then they compute their public values using their private values and the public value z. And these values should be 128 bit to make it secure enough against the attackers Lindell, (Y., & Katz. 2019).

The following steps explain how to get the shared key between Alice and Bob so first of all:

Alice generates his public value as:	Bob generate his public value as:
$A_1= (a_1 \vee a_2' \wedge z_1)$	$B_1 = (b_1 \vee b_2' \wedge z_1)$
$A_2= (a_2 \vee a_3' \wedge z_2)$	$B_2 = (b_2 \vee b_3' \wedge z_2)$
.	.
$A_{128}= (a_{128} \vee a_1' \wedge z_{128})$	$B_{128}= (b_{128} \vee b_1' \wedge z_{128})$

Then they exchange the generated public values.
Finally, Alice and Bob compute the same secrete key k. as shown it the following:

Alice receives the Bob's public key and computes k:	Bob receives the Alice's public key and computes k:
$K_1 = (b_1 \vee b_2' \wedge z_1) \wedge (a_1 \vee a_2')$	$K_1= (a_1 \vee a_2' \wedge z_1) \wedge (b_1 \vee b_2')$
$K_2 = (b_2 \vee b_3' \wedge z_2) \wedge (a_2 \vee a_3')$	$K_2= (a_2 \vee a_3' \wedge z_2) \wedge (b_2 \vee b_3')$
.	.
.	.
$K_{128}= (b_{128} \vee b_1' \wedge z_{128}) \wedge (a_{128} \vee a_1')$	$K_{128}= (a_{128} \vee a_1' \wedge z_{128}) \wedge (a_{128} \vee b_1')$

In this case both of Alice and Bob can get the same secret key. For example:

Alice gets

$$K_1 = (b_1 \vee b_2' \wedge z_1) \wedge a_1 \vee a_2' = (b_1 \vee b_2') \wedge z_1 \wedge (a_1 \vee a_2')$$

Bob gets

$$K_1= (a_1 \vee a_2' \wedge z_1) \wedge b_1 \vee b_2' = (a_1 \vee a_2') \wedge z_1 \wedge (b_1 \vee b_2')$$

This algorithm is secure against man in the middle attack. Both of Alice and Bob operate some Boolean operation on privet keys and send to each other's, so in this case the private keys are not shown. Also both of them compute the secret key from the privet keys and the received values from the other side, and the attackers cannot drive the secret key using the public values. That means the only way to get the secret key is getting the privet keys, which are not known.

3.4 The Proposed Framework

The goal of the proposed approach is to build a shared key from private keys and other information. Therefore, the suggested system only takes such values into account. To make the system easier to describe, the suggested technique employed the names Bob for the second participant and Alice for the first participant, as shown in figure (4).

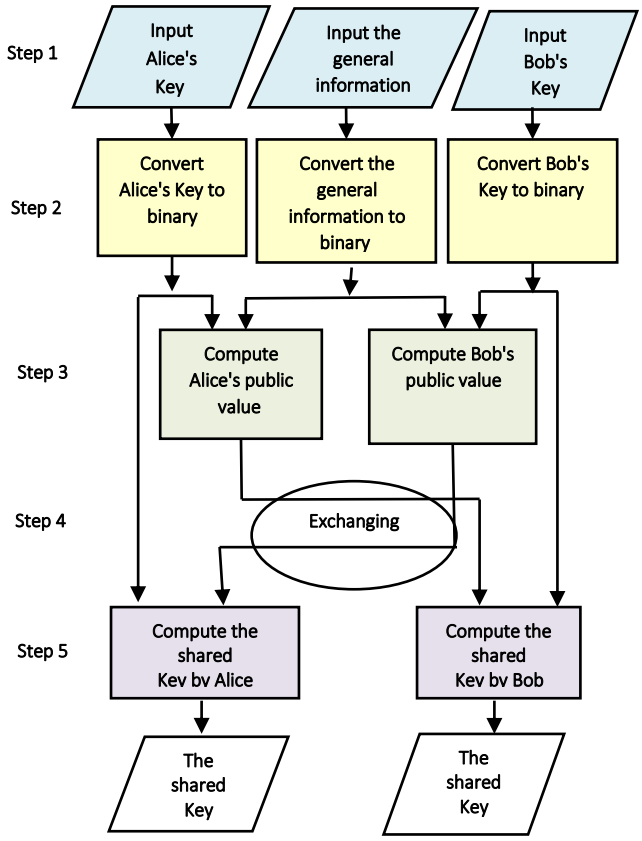

Figure (4). Flowchart to explain the proposed framework

The following steps explain clearly the processes that are followed in the system.

Step 1: First, entries such as Alice's private key, Bob's private key, and general information are sent to the system. Enter numbers, letters, or other characters. All activities are carried out using Boolean operations because the system is built on Boolean algebra, and as a result, all entries must be in binary format.

Step 2: All entries should be in binary format because the system's Boolean algebraic design requires it. The system transforms the entries into binary arrays in this stage to enable the Boolean operations. By submitting the entries to a conversion function, the system transforms them into binary format. The binary arrays are sent to the main programmer by the conversion function once it has converted the entries to binary format.

Step 3: The public values of both Alice and Bob are generated by the programmer in this stage using their respective private keys and the general data. In the beginning, the system determines Alice's public value by submitting his private key and other data to a function that determines public values. In this instance, it calculates Alice's public value before sending it to the primary programmer. The system next computes Bob's public value by providing his private key and other data to the same function that computed the public values in step two. In this instance, it calculates Bob's public value before sending it to the primary programmer.

3.5 The Project Functions

To accomplish the system's goals, some functions are created. To be specific, AND, OR, and NOT are the Boolean operations employed in this project. When the program receives the data—"the first participant private key, the second participant private key, and the general information"—it transforms the data to binary format and begins to carry out the functions. The first function computes the function for public values.

3.5.1 Computing the Public Values Function

This function, which is used in step 3, takes the data as binary arrays and computes the public values for each participant independently. In order to compute the first participant's public key, it must first receive both the general information and the first participant's private key, both of which are in binary format. The second participant's public key is then calculated after the second participant's private key and general information (both in binary format) are received.
Furthermore, the public value of each participant is determined independently. The main programmer then receives those public values and exchanges them in order to determine the shared key.

4 Results and discussion

The system design and the techniques employed to achieve the desired outcomes. In order to help readers have a deeper knowledge of the methods, the system's execution, and its outcomes, two issues with the proposed system are also highlighted: security and key size. There has also been a quick comparison between the offered methods and Daffier-Hellman.

4.1 Security of the Proposed Method

4.1.1 Key Size: Greater security is associated with larger keys. Key sizes of 128 bits or more are now the norm, and those of 64 bits or less are no longer regarded as sufficient. The key size in the suggested approach is 128 bits, which is deemed secure enough to withstand a brute force assault.

A cryptographic technique can be discovered through a brute force assault, which involves testing numerous options. In this instance, the attacker must attempt each potential key in order to find the shared key. Therefore, there is a 212 percent chance of a brute-force attack, which is currently seen to be secure enough.
In order to obtain the shared key, the attacker must attempt every key that might be used with one of the computed public values. The key size utilized is 128 bits, which means 2128 potential combinations.

4.1.2 The Design of the Equation: The Boolean satisfiability problem (SAT), a decision issue taken into consideration by complexity theory, was used in the construction of the functions. An illustration of an NP-complete problem is the Boolean satisfiability problem. The most challenging NP problems are those that are NP-complete because, if an algorithm could be developed to solve any NP-complete problem rapidly, it would then be easy to apply it to all NP problems. As a result, the equation is thought to be secure because it was built on an NP-complete problem.

Furthermore, even though the attacker has access to the general information, the first participant's private key, and the second participant's private key in the proposed method, they cannot compute the shared key because it is necessary to know the other participant's private key in order to obtain the shared key using one participant's public value. Which nobody else than the key holder is aware of.

4.1.3 The Randomness Checking: The equation should be designed with a few criteria in mind. Attempt to construct an equation with a reasonable avalanche probability, which states that a change in one bit of the input should result in a change in many bits of the output; a stricter version of this is the severe avalanche criteria (SAC). This specifies that any modification to any bit of the input should modify the probability of the output by a factor of two.
This requirement was attempted to be kept in our method, and the proposed approach has been evaluated to determine whether or not it is.

First, the second participant's private key was fixed and the general information. Next, the first participant's private key by flipping a bit in the first participant's private, and implemented the algorithm 128 times "the key size" while flipping a different bit in the first participant's private key each time. The number in use is:
10101010101010101010101010101010101010101010101010
10101010101010101010101010101010101010101010101010
10101010101010101010101010

Following the implementation of the method, the first result was compared to the other results, and the following graph was created as shown in Figure (5), depending on the quantity of altered bits.

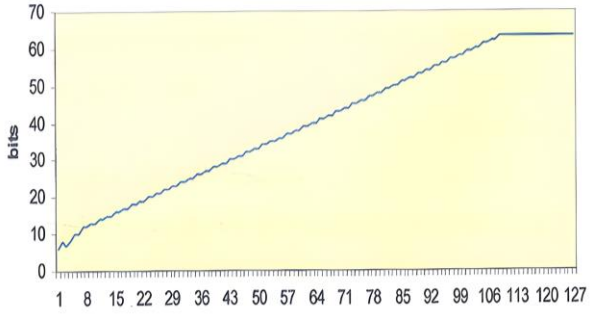

Figure (5). Distribution of the changing between the first result and the other results

Secondly, the first participant's and the second participant's private keys, assigned the same previous number to all three entries, and changed the general information by flipping a bit in it. Finally, implemented the algorithm 128 times "the key size" while flipping a different bit in the general information each time. Following the implementation of the method, the first result was compared to the other outcomes, and the following graph was created as shown in Figure (6), depending on the quantity of altered bits.

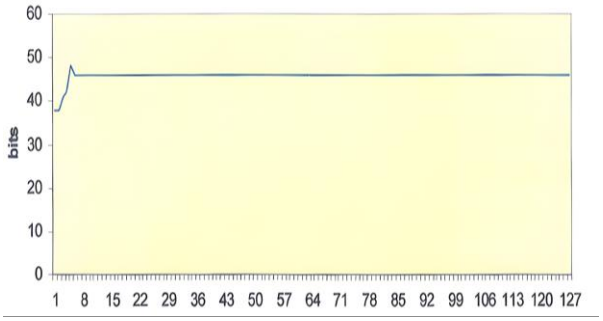

Figure (6). Distribution of the changing between the first result and the other results

The results for the two previous comparisons should be roughly 64, which is in the middle of the key size. But as evidenced, some adjustments need to be made to improve the outcomes.

4.2 Comparison with Diffie-Hellman

In some cases, a comparison between the proposed approach and Diffie-Hellman has been conducted.

4.2.1 Key Size: The key size in the Diffie-Hellman protocol is 256 bits, however in our suggested solution it is only 128 bits, which has become a standard size.

4.2.2 Running time: A variety of key sizes, including 46 bits, 128 bits, 256 bits, and 1024 bits, have been evaluated for both approaches' running times in the comparison. The test has been run numerous times for each key, the results average has been calculated, and the graph displayed in Figure (7) is then generated based on the results.

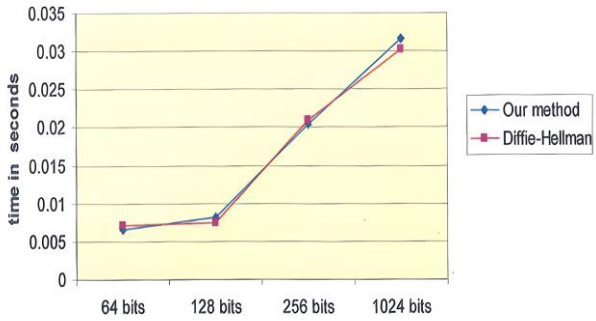

Figure (7). Comparing the running time with Diffie-Hellman

It is evident from the graph that there is not a significant difference between the running times of the two approaches. Both this approach and Diffie-Hellman can be faster on occasion.

However, so far, the key size for our suggested approach is 128 bits, whereas the key size for Diffie-Hellman is 256 bits. As can be seen in the graph, our method takes less time to run with a key size of 128 bits than Diffie-Hellman does with a key size of 256 bits. Therefore, the suggested method is thought to be faster than Diffie-Hellman.

4.2.3 Security: If p and g are properly selected, the Diffie-Hellman protocol is thought to be safe against attackers. To obtain gab, the assailants must be able to resolve the Diffie-Hellman puzzle. And that is regarded as challenging. The secret integers a and b are eliminated at the end of the session. Therefore, Diffie-Hellman key exchange alone provides security and effectively conceals the private keys.

The proposed method is thought to be secure because, in order to obtain the shared key, one must first know the private keys, which are impossible to obtain due to the Boolean satisfiability problem (SAT) concept. Even with knowledge of the computed public values, however, it is still impossible to obtain the shared key because no private keying material is available to be revealed.

The major objective is to introduce a novel Boolean algebraic technique for key exchange. This project meets the main conditions, which are: getting the same value for a shared key, by both the two participants, and obtaining that securely, i.e., even if the others acquired the public values, it is impossible to compute the shared key. According to the NP-hard problem, the security of this solution is attained.

Through communication channels, the cryptosystems are utilized to secure network information. Most cryptographic techniques use an echo of the technique's complexity, which is then used to produce a coded word or message that conceals the data. The major applications of a cryptosystem are key exchange, digital signatures, and encryption decryption.

These protocols make use of the NP-Hard problem difficulty to guarantee the security of the key exchange. In a Li and Zhang (2019) investigation, based on the Boolean satisfiability problem (SAT), the protocol for safe key exchange is suggested in this study. The two parties first create a set of random variables, which is how the protocol operates. They then create a SAT problem using these variables. Along with another Li and Zhang paper (2020), Based on the SAT problem, this study suggests an enhanced, secure key exchange system. The technique has been improved, using fewer random variables overall.

And another study by Singh and Babu (2018), Based on the knapsack problem (KP), a secure key exchange technique is suggested. Based on the KP, this study suggests an improved secure key exchange technique. The protocol has been improved in that it uses a KP problem-solving algorithm that is more effective.

Also the research by Vetrivel and Padmavathi (2019), Based on the graph coloring problem (GCP), a secure key exchange technique is suggested in this study. They create a GCP problem using these figures. The shared key is the answer to this GCP issue.

However, the complexity of the underlying NP-hard problem determines how secure the protocols will be.The effectiveness of the protocols depend on both numbers of random variables that used and the algorithm used to solve the underlying NP-Hard issue. For instance, the protocol proposed by Singh and Babu (2021) is a viable choice if the application requires a modest key size. If the application requires a high level of security, the protocol proposed by Li and Zhang (2019) is a possible choice.

The main objective of the proposed study is to safely get the same value for both participants as a shared key, even if others already know the public values. Higher security guarantees are provided by Boolean algebra and an NP-hard problem than by traditional methods that merely take into account computational complexity. The study used a 128-bit key size, which yields good results and provides greater security guarantees than traditional methods. The work has also produced a novel key exchange scheme that improves on existing techniques. Overall, this approach represents a significant improvement in the field of secure key exchange using Boolean algebra and NP-hard problems.

5 Conclusions and Recommendations

5.1 conclusions

The use of Boolean operations in cryptosystems, such as Boolean algebra to produce the public key and Boolean permutation, has been studied in considerable detail. A NP-hard problem, of which there are several different

varieties, has been employed in suggested methodology to create key exchange. One of these problems, the Boolean satisfiability problem, has been used. A Boolean expression that simply uses the variables AND, OR, and NOT serves as the solution to this problem. It is intended that when the expression is presented, the variables will have had their TRUE and FALSE values assigned, making the entire expression true. An approach has been suggested based on this notion, and the system has been created using that approach.

The suggested system utilizes the general knowledge and private keys of two participants to produce a public value. Following that, each participant uses his or her own private key along with the other public value to drive the shared key. Because the private keys are not displayed, this technique is regarded as being secure against man-in-the-middle attacks. Both of them compute the secret key using the private keys and the values they have received from the other side, making it impossible for attackers to drive the secret key using the public values. Therefore, obtaining the private keys, which are unknown, is the only method to obtain the secret key. The project is implemented, and positive outcomes are attained. Using a key with 128 bits makes the key secure; in fact, it is a size that is widespread at the moment.

5.2 Recommendations

The method can be enhanced in a few ways, such as getting a better equation to achieve better results and making it so that it has a good avalanche probability, which means that any change to the input should change the probability of the output by "half" if it occurs.

According to the NP-hard problem, it is conceivable to implement key exchange for more than only the two portions of this study. This paper focuses on key exchange based on the NP-hard issue, which is intriguing due to its difficulty and lack of usage in cryptosystems. As a result, this issue can be used to future work on digital signatures and encryption decryption.

The major goal of the suggested study is to use a shared key to accomplish the same value for both participants while remaining safe, even if others are aware of the public values. Better security guarantees are offered by NP-hard issues and Boolean algebra than by more conventional approaches that only consider computational complexity. The study's key size was 128 bits, which yields good results and provides greater security assurances than traditional methods. The work also led to the creation of a brand-new key exchange strategy that improves upon existing techniques. This approach represents a significant advancement in terms of secure key exchange using NP-hard problems and Boolean algebra.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Oscillation of Super-linear second Order Nonlinear Differential Equations with Damping Term

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A B S T R A C T

The study of differential equations has been the object of many researchers over the last decades. Different approaches and various techniques have been adopted to investigate the qualitative properties of their solutions. Recently and driven by their widespread applications, the investigation of differential equations of second order has drawn significant attention. The oscillation of solutions has been the main features that have attracted consideration. Therefore, it has been intended to use the Riccati Transformation Technique for obtaining several new oscillation criteria for different classes of nonlinear differential equations of the second order with a damping term. Oscillatory behavior has taken into account through this study of solutions of some differential equations. Comparisons between our results and the previously known results have presented. The relevance of our theorems has been clear due to carefully selected examples. As a conclusion, our aim is to provide some results to improve and/or extend some of well-known results in the literature.

1 Introduction

This paper concerned with oscillation of the solution to the damped ordinary differential equation of the form:

$$(r(t)\psi(x(t))x'(t))' + h(t)x'(t) + q(t)g(x(t)) = H(t, x(t), x'(t)), \quad (1)$$

where r, ψ, h and q are continuous functions on the interval $[t_0, \infty), t_0 \geq 0$ $r(t)$ is a positive function ,ψ is continuous function on the real line $\mathbb{R}$, with $\psi(x) > 0, \forall x \in \mathbb{R}$ and g is continuously differentiable function on the real line $\mathbb{R}$ except possible at 0 with $xg(x) > 0$ and $g'(x) \geq k > 0$for all $x \neq 0$. $H$is a continuous function on $[t_0, \infty) \times \mathbb{R}^2$ with $\frac{H(t,x(t)x'(t))}{g(x(t))} \leq p(t)$ for all $t\epsilon[t_0, \infty)$

Throughout this study, our attention is only to the solutions of the differential equation (1) that exist on some ray$[t_x, \infty)$ where t_x may depend on the particular solution.

In the past decades, the problems regarding the study of oscillation criteria of differential equations with damping have become an important area of research because such equations arise in many real life problems; see the research papers (Ayanlar & Tiryaki, 2000, Elabbasy & Elhaddad, 2007, Kirane & Rogovchenko, 2001, Mustafa et al., 2004, Nagabuchi &Yamamoto, 1988, Naito, 1974, S. & Rogovchenko, 2003, Rogovchenko &Tuncay, 2007, Rogovchenko &Tuncay, 2008, Rogovchenko & Tuncay, 2009, Saker et al.,2003, Tunc & Avci, 2012, Wang & Song, 2013, Xiaoling & Chenghui, 2013, Zhang & Song, 2011 and Zheng, 2006) and the references cited therein. In particular, second order-damped differential equations are used in the study of vehicle noise, vibration and harshness of vehicles (NVH). In what follows, we present the background details that motivate the contents of this paper.

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(Elabbasy *et al*. 2005, Lu & Meng, 2007, and Rogovchenko &Tuncay, 2008), established some new oscillation conditions of Kamanev and Philos type for the Eq. (1) with $\psi(x(t)) = 1, H(t, x(t), x'(t)) = 0$and they did not sign conditions on$h(t), q(t)$. Rogovchenko & Tuncay, (2009) considered Eq. (1) with $H(t, x(t), x'(t)) = 0$and Berkan (2008), continued the investigation of new oscillation of Eq. (1) but he put $H(t, x(t), x'(t)) = H(t)$.

Zhang & Song, (2011) considered equation (1) when replaced explicit function $q(t)g(x(t))$ by implicit function $Q(t, x)$ they obtained certain necessary criteria for oscillation of Eq. (1).

Wang & Song, (2013), established certain oscillation standards for Eq. (1), with $H(t, x(t), x'(t)) = 0, \frac{g(x(t))}{x(t)} \geq k > 0$ for $x \neq 0$.

Xiaoling & Chenghui, (2013), established an important extension of the celebrated oscillation criteria for (1), they studied it with $\psi(x(t)) = 1, g(x(t)) = g(x(\tau(t)))$ and$H(t, x(t), x'(t)) = 0$.

In the same way, we localize generalize Reccati technique to derive new oscillation conditions for Eq. (1). Our results are more general than the previous results. Precisely chosen examples are provided to demonstrate the influence of impulses on the oscillatory actions of all solutions in this class.

2 Definitions

Definition 1. A solution $x(t)$ of Eq. (1) is oscillatory if it has arbitrarily large zeros; otherwise, we call it non-oscillatory. The Eq. (1) is oscillatory if all of its solutions oscillate.

Definition 2. Equation (1) is said to be super-linear if

$0 < \int_{\pm\varepsilon}^{\pm\infty} \frac{du}{g(u)} < \infty$for every all $\varepsilon > 0$

3 Oscillation Results

In this section, I introduce some theorems that include new conditions for ensuring the oscillation of solutions equation (1).

Theorem 1: Suppose that

(1) $c_1 \leq \psi(x(t)) \leq c_2$ for all $x \epsilon \mathbb{R}$ and
(2) $h(t) \leq 0$for $t \geq t_0$hold.

Let ρ be a continuously differentiable positive function over$[T, \infty)$ such that $\rho'(t) \geq 0$

over $[T, \infty)$; $(\rho'(t)r(t))' \leq 0$and such that
(3) $\lim_{t\to\infty} \int_{T_0}^{t} \frac{1}{\rho(s)r(s)} ds = \infty$,
(4)
$\lim_{t\to\infty} \int_{T_0}^{\infty} R(s)ds = \infty; R(s) = \rho(s)[q(s) - p(s)] - \frac{1}{4A}\frac{(\rho'(s))^2}{\rho(s)} r(s)$,
A is constant.
Then, every solution of equation (1) is oscillatory.

Proof: Without loss of generality, we may hypothesize that there exists a solution $x(t)$ of equation (1) such that

$$x(t) > 0 on [T, \infty), for some T \geq t_0 \geq 0.$$

Define

$$\omega(t) = \frac{r(t)\psi(x(t))x'(t)}{g(x(t))}, t \geq T \quad (1)$$

For all $t \geq T_0$ then differentiating Equality (1) and using Eq. (1), we obtain

$$\left(\frac{r(t)\psi(x(t))x'(t)}{g(x(t))}\right)' \leq \frac{H(t, x'(t), x(t))}{g(x(t))} - q(t) - \frac{h(t)x'(t)}{g(x(t))} - \frac{r(t)\psi(x(t))x'(t)g'(x(t))x'(t)}{g^2(x(t))}$$

Since $g'(x(t)) \geq k$and using the condition (1) we have

$$\left(\frac{r(t)\psi(x(t))x'(t)}{g(x(t))}\right)' \leq -[q(t) - p(t)] - \frac{h(t)x'(t)}{g(x(t))} - \frac{kr^2(t)\psi^2(x(t))(x'(t))^6}{c_2 g^2(x(t))}, t \geq T \quad (2)$$

Multiplying the inequality (2) by $\rho(t)$ and integrate form T to t we obtain

$$\frac{\rho(t)r(t)\psi(x(t))x'(t)}{g(x(t))} \leq C_T - \int_T^t \rho(s)[q(s) - p(s)]\, ds - \int_T^t \frac{\rho(s)h(s)x'(s)}{g(x(s))} ds + \int_T^t \left[\rho'(s)\omega(s) - A\frac{\rho(s)}{r(s)}\omega^2(s)\right] ds \quad (3)$$

Where $C_T = \frac{\rho(T)r(T)\psi(x(T))x'(T)}{g(x(T))}$.

By using complement square, the inequality presented by (3) can be written as

$$\frac{\rho(t)r(t)\psi(x(t))x'(t)}{g(x(t))} \le C_T - \int_T^t \rho(s)[q(s)-p(s)]\,ds - \int_T^t \frac{\rho(s)h(s)x'(s)}{g(x(s))}\,ds$$

$$+\int_T^t \left[A\frac{\rho(s)}{r(s)}\left(W^2(s) - \left(\frac{\rho'(s)r(s)}{2A\rho(s)}\right)^2\right)\right] \quad (4)$$

By the Bonnet theorem, for a fixed $\varepsilon_t \in [T,t]$ such that

$$-\int_T^t \frac{\rho(s)h(s)x'(s)}{g(x(s))}ds = -\rho(T)h(T)\int_T^{\varepsilon_t}\frac{x'(s)}{g(x(s))}ds$$

Since$(-\rho(t)h(t)) \ge 0$ and the equation (1) is super-linear, we have

$$-\infty < \int_T^t -\rho(s)h(s)\frac{x'(s)}{g(x(s))}ds \le B, \quad (5)$$

where $B = -\rho(T)h(T)\int_{x(T)}^{\infty}\frac{du}{g(u)}$.

By (5) and the condition (4), (3) become

$$\frac{\rho(t)r(t)\psi(x(t))x'(t)}{g(x(t))} \le C_T + B_1 - \int_T^t R(s)\,ds$$

By the condition (4), we have

$$\lim_{t\to\infty}\frac{\rho(t)r(t)\psi(x(t))x'(t)}{g(x(t))} = -\infty.$$

Thus, there exists $T_1 \ge T$such that $x'(t) < 0$for all $t \ge T_1$ The condition (4) also implies that there exists $T_2 \ge T_1$such that

$$\int_{T_1}^{T_2}\rho(s)(q(s)-p(s))\,ds = 0\text{and}$$
$$\int_{T_2}^t \rho(s)(q(s)-p(s))\,ds \ge 0 \text{ for } t \ge T_2$$

Multiplying equation (1) by$\rho(t)$, from the definitions of the functions and condition (2), we get

$$\rho(t)(r(t)\psi(x(t))x'(t))' + \rho(t)h(t)x'(t) + \rho(t)q(t)g(x(t)) = \rho(t)H(t,x(t),x'(t))$$
$$\rho(t)(r(t)\psi(x(t))x'(t))' + \rho(t)g(x(t))q(t) \le \rho(t)g(x(t))p(t), t \ge T_2. \quad (6)$$

Integrate the inequality (6) from T_2 to t we obtain

$$\rho(t)r(t)\psi(x(t))x'(t) \le \rho(T_2)r(T_2)\psi(x(T_2))x'(T_2) + \int_{T_2}^t \rho'(s)r(s)\psi(x(s))x'(s)\,ds - g(x(t))\int_{T_2}^t \rho(s)(q(s)-p(s))\,ds + \int_{T_2}^t g'(x(s))x'(s)\int_{T_2}^s \rho(u)(q(u)-p(u))duds$$

By the condition (1) and the Bonnet's theorem, for $t \ge T_2$there exists $\gamma_t \in [T_2,t]$ such that

$$c_2\rho(t)r(t)x'(t) \le \rho(T_2)r(T_2)\psi(x(T_2))x'(T_2) + c_1\rho'(T_2)r(T_2)[x(\gamma_t)-x(T_2)] - g(x(t))\int_{T_2}^t \rho(s)(q(s)-p(s))\,ds + \int_{T_2}^t g'(x(s))x'(s)\int_{T_2}^s \rho(u)(q(u)-p(u))\,duds, t \ge T_2$$

Thus

$$c_2\rho(t)r(t)x'(t) \le \rho(T_2)r(T_2)\psi(x(T_2))x'(T_2), t \ge T_2$$

Dividing the last inequality by$\rho(t)r(t)$, integrate from $T_2$to t and the condition (3), we have

$$c_2x(t) \le c_2x(T_2) + \rho(T_2)r(T_2)\psi(x(T_2))x'(T_2)\int_{T_2}^t \frac{ds}{\rho(s)r(s)} \to -\infty$$

$as\ t \to \infty$, that is a inconsistency to the fact that $x(t) > 0$for $t \ge T$. This complete the proof.

Theorem 2: Suppose that the condition (1) hold, and

(5) $\int_T^{\infty}\frac{ds}{r(s)} \le k_1, k_1 > 0$

(6) $\int_{\pm\varepsilon}^{\pm\infty}\frac{\psi(u)du}{g(u)} < \infty forall\varepsilon > 0.$

Furthermore, suppose that there exists a positive continuous differentiable function ρ on the interval $[t_0,\infty)$ with $\rho(t)$ is a non-decreasing function on the interval$[t_0,\infty)$such that

(7)$\limsup_{t\to\infty}\int_T^t \frac{1}{r(s)\rho(s)}\int_T^s \rho(u)\left[q(u) - p(u) - \frac{h^2(u)}{4c_1kr(u)}\right]duds = \infty,$

where $\rho: [t_0,\infty) \to (0,\infty)$.

Thus, each solution of super-linear equation (1) is oscillatory.

Proof: Without loss of generality, we can suppose that there exists a solution $x(t)$of equation (1) such that $x(t) > 0 on [T,\infty) for some T \geq t_0 \geq 0$. Define

$$\omega(t) = \frac{\rho(t)r(t)\psi(x(t))x'(t)}{g(x(t))}, t \geq T$$

This and by the condition (1) and Eq. (1), we have

$$\omega'(t) \leq \rho(t)p(t) - \frac{\rho(t)h(t)x'(t)}{g(x(t))} - \rho(t)q(t) + \frac{\rho'(t)}{\rho(t)}\omega(t) - \frac{c_1 k\rho(t)r(t)(x'(t))^2}{g^2(x(t))}, t \geq T$$

Thus for $t \geq T$, we have

$$\rho(t)\left(\frac{\omega(t)}{\rho(t)}\right)' \leq \rho(t)p(t) - \rho(t)q(t) - \frac{\rho(t)h(t)x'(t)}{g(x(t))} - \frac{c_1 k\rho(t)r(t)(x'(t))^2}{g^2(x(t))}, t \geq T$$

$$\rho(t)[q(t) - p(t)] \leq -\rho(t)\left(\frac{\omega(t)}{\rho(t)}\right)' - \frac{\rho(t)h(t)x'(t)}{g(x(t))} - \frac{c_1 k\rho(t)r(t)(x'(t))^2}{g^2(x(t))}, t \geq T$$

Integrate from T to t, we obtain

$$\int_T^t \rho(s)[q(s) - p(s)]\,ds \leq \int_T^t -\rho(s)\left(\frac{\omega(s)}{\rho(s)}\right)' ds - \int_T^t \frac{\rho(s)h(s)x'(s)}{g(x(s))}ds - \int_T^t \frac{c_1 k\rho(s)r(s)(x'(s))^2}{g^2(x(s))}ds, \quad t \geq T$$

$$- \int_T^t \frac{\rho(s)h(s)}{g(x(s))} + \frac{c_1 k\rho(s)r(s)(x'(s))^2}{g^2(x(s))}ds = -\int_T^t \left[\sqrt{c_1 k\rho(s)r(s)}\frac{x'(s)}{g(x(s))} + \frac{1}{2}\sqrt{\frac{\rho(s)}{c_1 kr(s)}}h(s)\right]^2 ds + \frac{1}{4kc_1}\int_T^t \frac{\rho(s)h^2(s)}{r(s)}ds \leq \frac{1}{4kc_1}\int_T^t \frac{\rho(s)h^2(s)}{r(s)}ds$$

By the Bonnet's theorem, since $\rho(t)$ is a non-decreasing function on the interval$[t_0,\infty)$, there exists $T_1 \in [T,t]$such that

$$-\int_T^t \rho(s)\left(\frac{\omega(s)}{\rho(s)}\right)' = -\rho(t)\int_{T_1}^t \left(\frac{\omega(s)}{\rho(s)}\right)' ds = -\rho(t)\int_{T_1}^t \left(\frac{d}{ds}\frac{\omega(s)}{\rho(s)}\right)ds = -\rho(t)\frac{\omega(t)}{\rho(t)} + \rho(t)\frac{\omega(T)_1}{\rho(T_1)} \quad (7)$$

From the inequalities (7) and (5) in the inequality (4), we have

$$\int_T^t \rho(s)\left[q(s) - p(s) - \frac{h^2(s)}{4c_1 kr(s)}\right]ds \leq -\rho(t)\frac{\omega(t)}{\rho(t)} + \rho(t)\frac{\omega(T)_1}{\rho(T_1)}.$$

$$\int_T^t \rho(s)\left[q(s) - p(s) - \frac{h^2(s)}{4c_1 kr(s)}\right]ds \leq -\omega(t) + \rho(t)\frac{\omega(T)_1}{\rho(T_1)}.$$

$$\int_T^t \rho(s)\left[q(s) - p(s) - \frac{h^2(s)}{4c_1 kr(s)}\right]ds \leq -\omega(t) + \rho(t)\frac{\omega(T)_1}{\rho(T_1)}.$$

Integrating the last inequality divided by $\rho(t)r(t)$ from T to t, taking the limit superior on both sides and by conditions (5) and (6), we have

$$\limsup_{t\to\infty} \int_T^t \frac{1}{r(s)\rho(s)}\int_T^s \rho(u)\left[q(u) - p(u) - \frac{h^2(s)}{4ckr(s)}\right]duds$$

$$\leq \limsup_{t\to\infty}\left\{\left(\frac{\omega(T_1)}{\rho(T_1)}\right)\int_T^t \frac{ds}{r(s)} - \int_{x(T)}^{x(t)}\frac{\psi(u)du}{g(u)}\right\}$$

$$< \infty \quad ast \to \infty,$$

which contradicts to the condition (7). Hence, the proof is completed.

4 Discussion

A set of new oscillation conditions are stated and proved which extend and improve previous oscillation criteria and cover the cases which are not covered by known results. Some of illustrative examples are provided to show the applications of the oscillation criteria and the comparisons between our results and previous results in the literature.

Example1: Consider the following differential equation

$$\left(\frac{1}{t}\left(\frac{x^6(t)+2}{x^6(t)+1}\right)x'(t)\right)' - t^2x'(t) + \left(t+\frac{\sin t}{t}\right)x^5(t) = \frac{2x^{12}(t)\sin t\cos(x'(t)+1)}{(x^7+1)t^3}, t \geq \frac{\pi}{2}$$

Here,

$r(t) = \frac{1}{t}, h(t) = -t^2, q(t) = t + \frac{\sin t}{t}, g(x) = x^5,$
$H(t, x(t), x'(t)) = \frac{2x^{12}(t)\sin t\cos(x'+1)}{(x^7+1)t^3},$

for all$x \neq 0$and $t > 0$.

$$\frac{H(t,x(t),x'(t))}{g(x(t))} = \frac{2x^{12}(t)\sin t\cos(x'+1)}{(x^7+1)t^3} \times \frac{1}{x^5(t)} \leq \frac{2}{t^3} = p(t)$$

for all$x \neq 0$and $t > 0$. $\psi(x) = \frac{x^6+2}{x^6+1}$ and

$1 \leq \psi(x) \leq 2$ for all $x \in \mathbb{R}$,
$\rho(t) = t, \rho'(t)r(t) = \frac{1}{t} > 0, (\rho(t)h(t))' = -3t^2 < 0$
$and (\rho'(t)r(t))' = \left(\frac{1}{t}\right)' = \frac{-1}{t^2} < 0 forall t > 0.$
So, can note that

$$\int_{t_0}^{\infty} \frac{ds}{\rho(s)r(s)} = \int_{t_0}^{\infty} ds = \infty,$$

$$R(s) = s^2 + \sin s - \frac{2}{s^2} - \frac{1}{4As^2}$$
$$\int_{t_0}^{\infty} R(s)ds = \infty.$$

All conditions of Theorem 1 are satisfied; thus, the given equation is oscillatory.

Example2: Consider the following differential equation

$$\left(\frac{(x^2(t)+2)}{t^4(x^2(t)+1)}x'(t)\right)' + \frac{x'(t)}{t^5} + t^4x^5(t) = \frac{x^5(t)\cos(x(t))}{t^9}, t > 0.$$

We note that

$r(t) = \frac{1}{t}, \ \psi(x) = \frac{x^2(t)+2}{x^2(t)+1} > 0 \ and 1 \leq \psi(x) \leq 2,$

for all $x \in \mathbb{R}, h(t) = \frac{1}{t^5}$, and $\frac{H(t,x(t),x'(t))}{g(x(t))} = \frac{\cos(x(t))}{t^9}$
$\leq \frac{1}{t^9} = p(t) forall \ t > 0 \ and \ x \neq 0.$

Let $\rho(t) = t^6$such that

$$\limsup_{t\to\infty} \int_T^t \frac{1}{r(s)\rho(s)} \int_T^s \rho(u)\left[q(u) - p(u) - \frac{h^2(u)}{4c_1kr(u)}\right]duds$$
$$= \limsup_{t\to\infty} \int_T^t \frac{1}{s^2} \int_T^s u^6\left[u^4 - \frac{1}{u^9} - \frac{1}{4c_1ku^6}\right]duds$$
$$= \limsup_{t\to\infty} \left[\frac{s^{10}}{110} - \frac{1}{6s^3} + \frac{1}{8c_1kTs^2} + \left(\frac{T^{11}}{11s} + \frac{1}{2sT^2} + \frac{1}{4c_1ksT}\right)\right]_T^t = \infty.$$

All conditions of Theorem2 are satisfied and hence each solution of the given equation is oscillatory.

Remark1: Theorem1 and Theorem 2 extend and improve results of (Elabbasy & Elzeine, 2011, Remili, 2008, and Results of Xhevair & Elisabeta, 2014).

Remark2: Remili, (2008) has established some oscillation results for Eq. (1) with $\psi(x(t)) = 1, h(t) = 1$, these results required that $r(t) \leq a_1$ and

$$\liminf_{t\to\infty} \int_T^t Z(s)ds > -\lambda, \quad (\lambda > 0)$$

for all large $T; Z(s) = R(s)[q(s) - p(s)]$, which are not required in Theorem 2.

5 Conclusions

To sum up, a set of new oscillation conditions are stated and proved which extend and improve previous oscillation criteria and cover the cases which are not covered by known results. Further, we introduced some illustrative examples. Remarks were also included to show the evidence of our main results.

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Maleic Acid Separation from Aqueous Solutions Utilizing Amberlite LA-2

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A B S T R A C T

This study investigates the potential uses of Amberlite LA-2 for the recovery of maleic acid from an aqueous solution. The effects of the initial concentration of maleic acid and Amberlite LA-2, pH, contact time, and temperature were determined and evaluated. The experimental results of extraction were used to calculate the distribution coefficient (K_D), and extraction efficiency (E%). The results show that the extraction efficiency increased with the increase in the initial concentration of maleic acid, where the percentage increased from 92.65% to 99.01% when the concentration of acid was increased from 0.01 to 0.075 N. The percentage of maleic acid extraction was also increased from 85.3 to 98% with the increase in the concentration of Amberlite LA-2 from 0.044 to 0.22 M. The acid extracted from the aqueous phase to the organic phase increases with time, and the quantitative transfer of maleic acid occurred after 60 minutes. At a concentration of 0.05 M, the percentage of extracted acid was observed to increase from 98.8% to 99.95% when temperature was increased from 25 to 40 °C. The highest percentage of acid extraction was recorded at pH=3, which is (90.3%). The maximum loading modulus reached a value of 1.6877 at a concentration of 0.044 M of the secondary amine.

1 Introduction

One of the most important methods in the science of separation is the application of liquid-liquid extraction (Blumberg, 1988; Raynie, 2000). This application requires ion exchange materials that differ from conventional materials not only in their functional groups but also in their physical form (Nasef, 2012). The use of water-insoluble acid-base and liquid reagents as liquid ion exchangers was first proposed by Smith and Page (Smith & Page, 1948; Werner, 1974). Since then, there has been a growing interest in using bases and high molecular weight acids as extracts (Kunin & Winger, 1962; Werner, 1974). As far as the importance of 'liquid exchangers' is concerned, all high molecular weight amines have been named liquid anionic exchangers (Khopkar, 2007). The use of ion exchange resins in the adsorption process by ion exchange is more efficient(Lo, Baird, & Hanson, 1991; Nasef, 2012). For the separation of carboxylic acids, the resins used are mostly strong or weak base resins, which contain tertiary or quaternary amines as the ion exchange group (Dethe, Marathe, & Gaikar, 2006). The carboxylic acids are usually recovered from ion exchange resins by rinsing with sodium hydroxide and can be concentrated through evaporation and then hydrolysis to produce a pure acid for further processing (urali, 2017). These amines may be primary, secondary, tertiary, or quaternary, depending on the number of hydrogens being replaced by the R-type groups to form the compound. For example, if R_3NH^+ is a tertiary amine and it undergoes a reaction with the anion as the following:

$$(R_3NH^+) + X^- \rightarrow (R_3NH^+X^-) \qquad (1)$$

Then, an uncharged complex is formed. This amine salt, in the presence of an anionic compound for example (Y), wıll undergo anion exchange as:

$$(R_3NH^+X^-) + Y^- \rightarrow (R_3NH^+Y^-) + X^- \qquad (2)$$

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The entire product is called "liquid anion exchangers" (Khopkar, 2007). The liquid-liquid extraction method is very useful in recovering compounds from mixtures of materials; the principle of extraction depends on the factor of distribution of materials between two immiscible liquids. A part of the substance will be transferred to the second solvent, forming two layers of two unmixed liquids, where each of them contains a certain percentage of the substance (Blumberg, 1988; Ricci, 1980). When the extraction process reaches equilibrium, i.e. after shaking and stabilization, the concentration proportion of the extracted substance in the organic and aqueous phases is a constant, and this is known as the distribution coefficient K_D. Extraction of an organic acid (HA) with a secondary amine (R_2NH) can be described by the following reaction:

$$HA + *R_2NH \rightarrow *(HA)\cdot(R_2NH) \qquad (3)$$

Where; HA represents the undissociated portion of the acid present in the aqueous phase while species in the organic layer are marked with an asterisk (*).

Amberlite LA-2 (N-Lauryl (trialkylmethyl) amine) is a liquid secondary amine of high molecular weight and is soluble in most common (non-polar) organic solvents (A. AL-abbasi, Alammarwy, A., & Abdulljaoad, S., 2022; A. Al-abbasi, Emmhemad, H., Suliman, H., 2022). Amberlite LA-2 is insoluble in water making it of remarkable interest in a wide range of different applications such as aqueous mineral separation processes in wastewater treatment and food processing industriery. Hydrometallurgy is one of the main applications of Amberlite in the field of mineralogy, it has been mainly used in the recovery and purification of uranium mainly from low-concentration ore as well as separating iron, cobalt and nickel in hydrochloric acid solutions (A. Al-abbasi & Kassim, 2011; A. Al-abbasi, Tan, & Kassim, 2010; Belkher, 2019; Lo et al., 1991; Raynie, 2000). Numerous organic acids, such as L(+) tartaric acid, formic acid, propionic acid, picric acid, malic acid, fumaric acid, glycolic acid (A. Al-abbasi, Emmhemad, H., Suliman, H., 2022; Y. S. Aşçı, and Ismail, I., 2009) (H. Uslu, Bayat, Gökmen, & Yorulmaz, 2009), (Kloetzer, 2019), (H. Uslu, 2016), (H. Uslu, and & Kırbaşlar, 2010), (Khopkar, 2007; Kloetzer, 2019) and levulinic acid and malic acid (H. Uslu, Datta, Santos, & Öztürk, 2019) were purified using Amberlite LA-2.

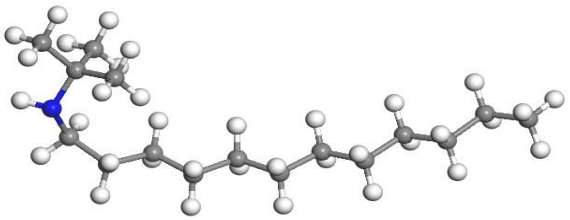


Figure 1: The chemical structure of Amberlite LA-2

Maleic acid, maleic anhydride, and fumaric acid (Figure 1) are multifunctional chemical intermediates that find applications in every field of industrial chemistry (Wiley). Each molecule contains two carbonyl acid groups and a double bond at the position. Maleic anhydride and maleic acid are raw ingredients in the production of resins, coatings, lubricants, copolymers, and ink production (Khalifa, 2018; Wojcieszak et al., 2015). Maleic acid can be formed by hydrolization of maleic anhydride. Isomerization of maleic acid produces fumaric acid which is a trans isomer of maleic acid concerning the double bond in the chemical molecule (Wiley).

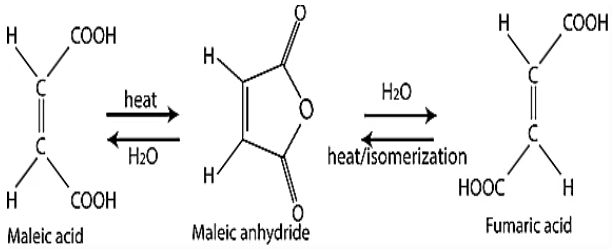


Figure 2: The chemical structure of maleic acid, fumaric acid, and maleic anhydride.

Moreover, in a recently published work by our group, high extraction ratios of about 97.65% and 99.9% respectively were obtained when Methyl Orange and Congo Red Dyes were removed by using Amberlite LA-2 (A. AL-abbasi, Alammarwy, A., & Abdulljaoad, S., 2022; A. AL-abbasi, Ehwedi, A., Ahmida, K., and Saleh,F., , 2023). Therefore, this study aims to investigate the extraction of maleic acid from Amberlite LA-2 by optimizing the extraction process in terms of the; initial acid and Amberlite LA-2 concentration, contact time, pH finally temperature. Furthermore, the mechanics of the extraction process of maleic acid with an anion exchanger in aqueous solutions were studied.

2 Experimental Section

2.1. The Chemicals and Instruments are used

The chemicals used are all of a high degree of purity and are produced by well-known companies. The type of chemicals used are; Maleic acid (Park Scientific Limited U.K, 99.8%), Amberlite LA-2 (BDH Laboratory Reagents, 99 %), Dichloromethane (Pure Chemistry, 99.8%), Sodium hydroxide (Analytical Reagent, 98%), Hydrochloric acid (Chemsolute, 37%), Sodium carbonate (Analytical Reagent, 99.8%), pH device (Thermo Electron/orion 3 star/USA) and water bath.

2.2. Preparation of Standard Solutions

Standard maleic acid solutions (0.1 N) were prepared as a stock solution by dissolving the required amount of 11.607g in a standard volumetric flask (volume 1 L). The volume was filled to the mark with distilled water. A solution of hydrochloric acid (0.1M) was prepared by taking the desired volume of concentrated acid 36 % in a standard volumetric flask 1L volume of distilled water and the acid concentration was adjusted by titrating using 0.1M sodium bicarbonate. The sodium hydroxide solution (0.1M) was prepared by dissolving 4g in volume 1L distilled water then the resulting solution was titrated with a standard solution of 0.1M hydrochloric acid. Different concentrations (0.044, 0.133, and 0.22 M) of Amberlite LA-2 were prepared in a 50 ml beaker by taking (1, 3, 5 mL) of liquid Amberlite and the volumes were diluted with dichloromethane solvent (DCM), the prepared solutions were simultaneously used on the same day to avoid evaporation of the solvent.

2.3 Extraction Experiment

Two equal quantities (10 mL each) of the previously prepared organic (DCM +Amberlite LA-2) and aqueous layers (tartaric acid) are taken in a 1:1 ratio and placed in tightly sealed containers. The mixture was shaken (200 rpm) in a water bath at desired temperature and time. A separating funnel was used to separate the aqueous layer from the organic layer after reaching equilibrium in each experiment. The lower organic layer is withdrawn from the separating funnel, then 4 ml of the aqueous layer is withdrawn for titration with sodium hydroxide (0.1 N), in the presence of phenolphthalein as the indicator to determine the amount of the remaining maleic acid in the aqueous phase. In addition, the extraction process was studied in the absence of Amberlite, so that 10 ml of two different concentrations of an aqueous solution of maleic acid 0.05M and 0.025M were taken separately then placed in an airtight bottle and 10 ml of dichloromethane (DCM) was added so far next step is shaken for 30 minutes at a temperature of 25°C. Experiments were carried out three times and their arithmetic average was taken. The distribution coefficients (K_D), extraction yields (E%) and loading factors (Z) can be determined using the below equations ;

$$K_D = \frac{[HA]_{org}}{[HA]_{aq}} \quad (4)$$

$$E\% = \left(1 - \frac{[HA]_{aq}}{[HA]_{0,\,aq}}\right) X\ 100 \quad (5)$$

$$Z = [HA]_{org} / [R_2NH]_o \quad (6)$$

Where $[Dye]_{org}$ and $[Dye]_{aq}$ are the dye concentration in the organic layer and the aqueous layer, respectively, and $[R_2NH]_0$ is the initial concentration of Amberlite LA-2. (A. AL-abbasi, Ehwedi, A., Ahmida, K., and Saleh,F., , 2023; A. Al-abbasi, Emmhemad, H., Suliman, H., 2022; H. Uslu, Baykal, Gök, Kırbaşlar, & Santos, 2020).

3 Results and Discussion

The distribution coefficients (K_D) and loading coefficient (Z) were studied and the percentage of extraction (E%) of the acid between the aqueous and organic phases to the organic class. The initial concentration of the acid and the concentration of the extractive substance (Amberlite LA-2), temperature, contact time, and finally pH were explored as the parameters influencing the extraction process. The experimental results of the extraction were compiled in Tables 1, 2, 3, and 4, which are listed in that order.

According to the collected data in Tables (1 and 2), the values of the distribution coefficients are typically higher than 1.0. This is explained by the fact that acid is distributed more widely in the organic phase than in the aqueous phase. The calculated distribution coefficient is displayed in Tables (1, 2, 3, and 4). It can be seen that all of the distribution coefficients increase as the initial acid concentration is raised from 0.1 to 0.075 M, demonstrating that the starting acid concentration has some positive effects on the distribution coefficient.

According to the results presented in Table (3), the greatest value of KD was 100 after an hour of combining 0.075 M and 0.044 M of maleic acid with an Amberlite AL-2, respectively. The high value of KD indicates that there is more maleic acid present in the organic phase than there is in the aqueous phase.

In general, the research results indicated Tables 1, 3, and 4 suggested that the loading factor would rise as the initial concentration of maleic acid did. However, as the concentration of Amberlite-LA2 declines, the loading percentage increase

3.1 The Effect of the Initial Concentration

In order to investigate the effect of the initial concentration of maleic acid on extraction efficiency %, maleic acid solutions with varied beginning concentrations in the range of 0.01–0.075 N were studied under circumstances of constant Amberlite–LA2 concentration, temperature, and pH. At 0.044 M of Amberlite-LA2 concentration, 25 °C, pH= 3.06, and various time intervals, the results are displayed in Table (2) and Figure (3). The overall findings show that as the initial acid maleic content increased, the extraction efficiency increased similarly.

Table 1: The results of the extraction of maleic acid induce the effect of the concentrations of the extracted and extracted substance.

C_{LA-2}	C_{HA}	t (min)	C_{aq}	C_{org}	K_D	Z	E%
0.044	0.01	15	0.00075	0.00926	12.51351	0.2104	92.6
		30	0.000375	0.009625	25.67	0.2187	96.25
		45	0.00025	0.00975	39	0.2215	97.5
		60	0.0002	0.0098	49	0.2227	98
	0.025	15	0.001388	0.023613	17.01802	0.5366	94.45
		30	0.000625	0.024375	39	0.5539	97.5
		45	0.000375	0.024625	65.66667	0.5596	98.5
		60	0.00025	0.02475	99	0.5625	99
	0.05	15	0.001225	0.048775	39.81633	1.1085	97.55
		30	0.000813	0.049188	60.53846	1.1179	98.375
		45	0.0007	0.0493	70.42857	1.1204	98.6
		60	0.0004	0.0496	124	1.1272	99.2
	0.075	15	0.00105	0.07395	70.42857	1.6806	98.6
		30	0.000775	0.074225	95.77419	1.6869	98.9666
		45	0.000825	0.074175	89.90909	1.6857	98.9
		60	0.000738	0.074263	100.6949	1.6877	99.0166
0.133	0.01	15	0.00113	0.00887	7.849558	0.0666	88.7
		30	0.000963	0.009038	9.38961	0.0679	90.375
		45	0.00085	0.00915	10.76471	0.0687	91.5
		60	0.000863	0.009138	10.5942	0.0687	91.375
	0.025	15	0.001888	0.023113	12.24503	0.1737	92.45
		30	0.001175	0.023825	20.2766	0.1791	95.3
		45	0.001083	0.023918	22.09469	0.1798	95.67
		60	0.001313	0.023688	18.04762	0.1781	94.75
	0.05	15	0.001563	0.048438	31	0.3641	96.875
		30	0.001238	0.048763	39.40404	0.3662	97.525
		45	0.001288	0.048713	37.83495	0.3662	97.425
		60	0.0015	0.0485	32.33333	0.3646	97
	0.075	15	0.00225	0.07275	32.33333	0.5469	97
		30	0.00175	0.07325	41.85714	0.5507	97.6666
		45	0.001713	0.073288	42.79562	0.5510	97.7166
		60	0.0017	0.0733	43.11765	0.0551	97.7333
0.22	0.01	15	0.001713	0.008288	4.839416	0.0376	82.875
		30	0.0011	0.0089	8.090909	0.0404	89
		45	0.001375	0.008625	6.272727	0.0392	86.25
		60	0.001463	0.008538	5.837607	0.0388	85.375
	0.025	15	0.0025	0.0225	9	0.1022	90
		30	0.001463	0.023538	16.09402	0.1069	94.15
		45	0.00155	0.02345	15.12903	0.1065	93.8
		60	0.001575	0.023425	14.87302	0.1064	93.7
	0.05	30	0.0031	0.0469	15.12903	0.2131	93.8
		30	0.002325	0.047675	20.50538	0.2167	95.35
		45	0.001716	0.048283	28.12621	0.2194	96.5666
		60	0.001525	0.048475	31.78689	0.2203	96.95
	0.075	15	0.003337	0.071662	21.47191	0.3257	95.55
		30	0.002437	0.072563	29.76923	0.3298	96.75
		45	0.002312	0.072687	31.25806	0.3303	96.9
		60	0.002313	0.072688	31.43243	0.3304	96.9166

Table 2: Maleic acid extracting in the absence of Amberlite LA-2

C_{LA-2}	C_{HA}	t(min)	C_{aq}	C_{org}	K_D	E%
0	0.01	30	0.0025	0.0075	3	75
	0.025	30	0.0053125	0.0196875	3.705882	78.75
	0.05	30	0.01	0.04	4	80
	0.075	30	0.011625	0.063375	5.451613	84.5

Table 3: The effect of pH on Maleic acid extraction

C_{LA-2}	C_{HA}	t (min)	pH	C_{aq}	C_{org}	K_D	Z	E%
0.133	0.01	30	1.92	0.0025	0.0075	3	0.0563	75
			3.06	0.000963	0.009038	9.38961	0.0679	90.375
			5.76	0.001	0.009	7	0.0676	87.5
			7.34	0.002	0.008	4	0.0601	80
			11.25	0.00375	0.00625	1.667	0.0469	62.5

Table 4: The effect of temperature on Maleic acid extraction.

C_{LA-2}	C_{HA}	t (min)	C_{aq}	C_{org}	K_D	Z	E%
0.044	0.025	15	0.0006	0.0244	40.6666	0.5545	97.6
		30	0.000138	0.024863	180.818	0.5650	99.45
		45	0.000025	0.024975	999	0.5676	99.9
		60	0.0003	0.0247	82.3333	0.5613	98.8
0.044	0.05	15	0.000075	0.049925	665.666	1.1346	99.85
		30	0.000025	0.049975	1999	1.1357	99.95
		45	0.0012	0.0488	40.6666	1.1090	97.6
		60	0.000275	0.0497	180.818	1.1295	99.45

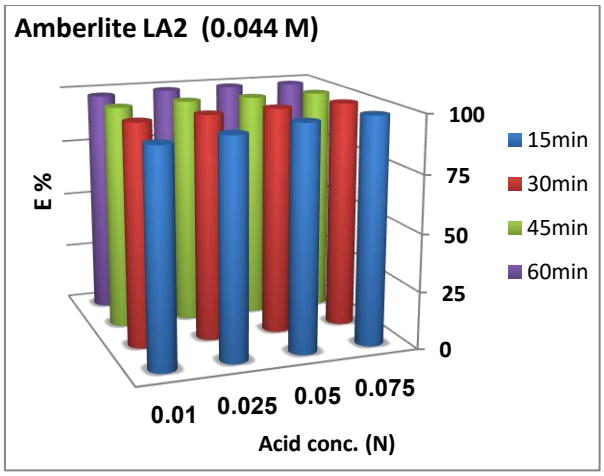


Figure 3: The effect of the initial concentration

3.2 Effect of Extractive Concentration (Amberlite LA-2)

To verify the role of the organic solvent in the extraction process, an extraction experiment was conducted in the absence of the extracting substance (secondary amine). From the results of the experiments shown in Figure (4), it was found that there is an amount of maleic acid above 75% that was extracted from the aqueous phase to the organic phase, due to the unlimited solubility of maleic acid in the DCM solvent, which is depended on to the carbon chain in the acid.

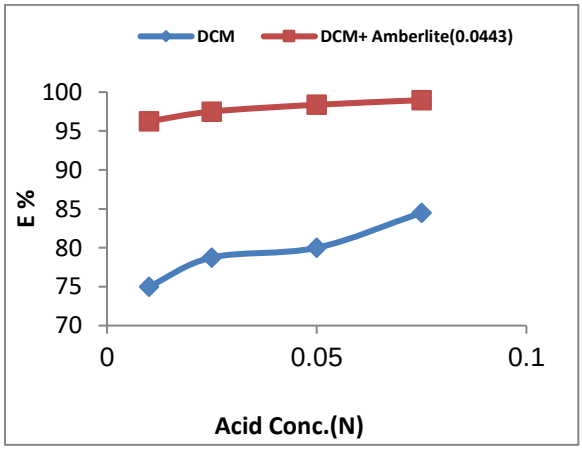
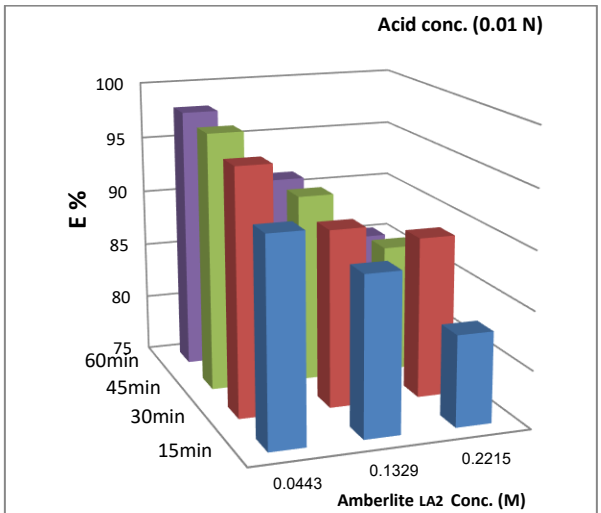


Figure 4: Extraction of maleic acid in the absence of the extract Amberlite LA-2

The effect of different concentrations of secondary amine (Amberlite AL-2) on the extraction of maleic acid was studied, where the varying concentrations of the extract were 0.044, 0.133, 0.22 M at room temperature (25ºC) and pH = 3.06. A relationship was drawn between the secondary amine concentration and the percentage of extraction at different times (15, 30, 45, and 60 minutes) as shown in Figure (5). It was observed that the percentage of maleic acid extraction increased from 85.3 to 98% with an increase in the extract concentration from 0.044 to 0.22 M.



	0.0443	0.1329	0.2215
15min	92.6	88.7	82.875
30min	96.25	90.375	89
45min	97.5	91.5	86.25
60min	98	91.375	85.375

Figure 4: Effect of Amberlite LA-2 concentration on the extraction process

3.3 Effect of pH on the Extraction Process

The pH of the acid solution was adjusted to a range of 2 to 11. The initial maleic acid concentration and Amberlite AL-2 concentration were both held constant, and the solutions' temperature was maintained at 25 °C

the maximum maleic acid extraction efficiency, 90.3%, was achieved at pH 3, as illustrated in Figure 5. In order to transfer the solute into the organic phase, the pH of the aqueous solution was therefore appropriate. With an increase in pH, carboxylic acid will dissociate more readily in an alkaline medium, and the organic phase will be able to extract less acid. The competition between the acid anion and the OH- ion for binding sites as a consequence of the presence of the OH- ion in the base solution can be used to explain why the extraction percentage has decreased.

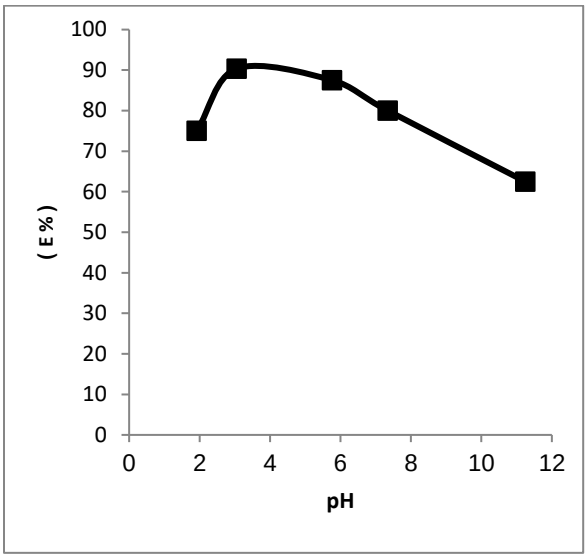


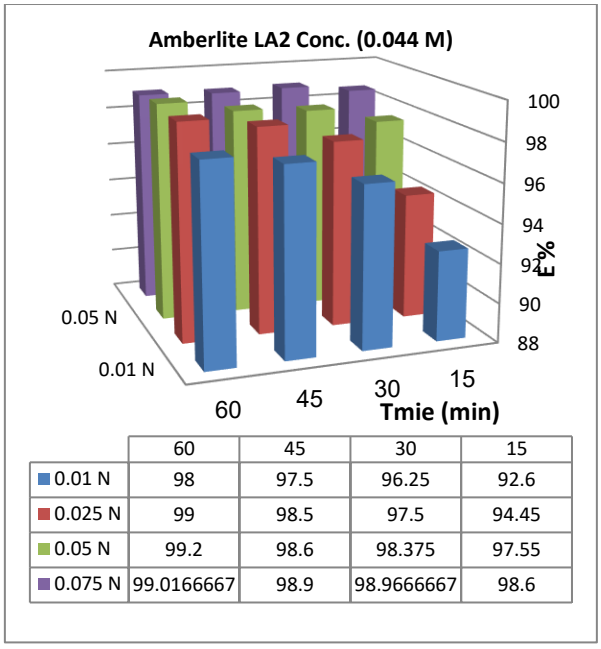
Figure 5: The effect of pH on the percentage of extraction

3.4 Effect of Contact Time

The purpose of this experiment was to determine the time required to reach equilibrium and also to study kinetics. The contact time was varied at 15, 30, 45, and 60 minutes, and the results are shown in Table 1. The results implied that the concentration of acid extracted from the aqueous phase to the organic phase increases with time, and the quantitative transfer of maleic acid took place after 60 minutes (Figure 6). At initial concentration of 0.01 M, the percentage of extraction increased from 92 to 99% when the contact time was prolonged from 15 to 60 minutes.

3.5 Effect of Temperature

The impact of temperature on the extraction of maleic acid from the aqueous phase at two different initial concentrations (0.025 and 0.05 M) was investigated. At a constant extractant concentration of 0.044 M and a fixed contact time of 30 min, the temperature was changed within 25 °C, 30 °C, and 40 °C. According to Figure 7, the proportion of extracted maleic acid with an initial concentration of 0.05 M appeared increase from 98.8% to 99.95% with increasing temperature. The extraction process is an activating process that increases with rising temperature, which is responsible for an increase in the extracted amount of maleic acid.



	60	45	30	15
0.01 N	98	97.5	96.25	92.6
0.025 N	99	98.5	97.5	94.45
0.05 N	99.2	98.6	98.375	97.55
0.075 N	99.0166667	98.9	98.9666667	98.6

Figure 6: The effect of contact time on the percentage of extraction

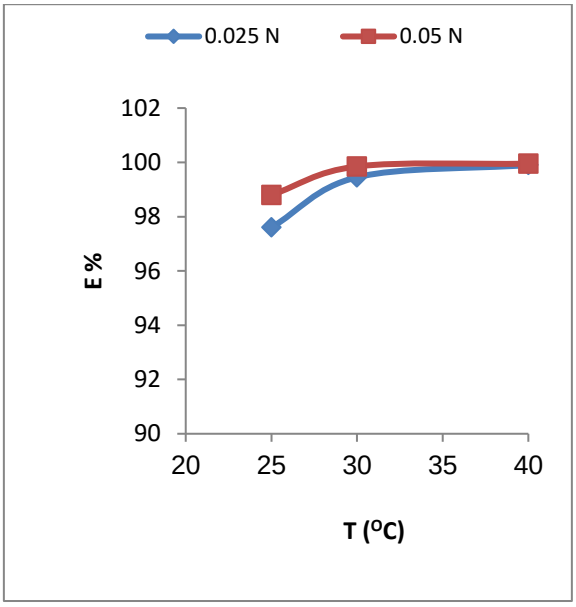


Figure 7: The effect of temperature on the extraction process

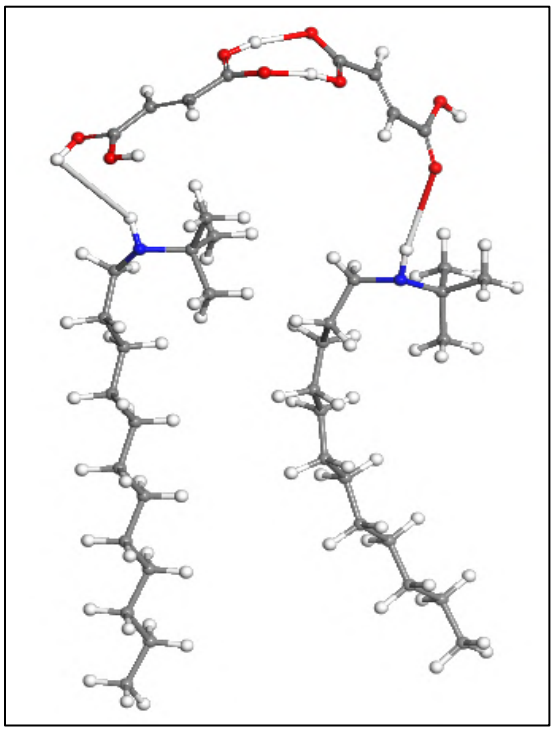


Figure : 8 The complex produced by the interaction of maleic acid and Amberlite LA-2

3.6 Extraction Process Mechanics

Based on research by Winestrin, the mechanism of complex formation is taken out to explain how acid is captured in the organic phase (Wennersten, 1983). To ascertain the molecular structure of the amine and acid mixture in the organic layer, Winestrin proposed that an ion pair forms once the acid and amine mix instantly. As shown in Figure 8, a compound is formed once the [OH] group of one acid molecule hydrogen bonds with the [C=O] group of the carboxyl group of another acid (Y. S. Aşçı & İnci, 2009; Y. S. Aşçı & İnci, 2009).

4 Conclusion

Maleic acid was successfully extracted from the aqueous solution via liquid/liquid extraction process using Amberlite LA-2 dissolved in DCM as an organic solvent. The distribution coefficients of maleic acid for the extraction process had values greater than 1, indicating a higher distribution of acid in the organic layer than in the aqueous layer. The distribution coefficient increased with increasing concentration of maleic acid in the medium. The results show that the extraction efficiency increased with the increase in the initial concentration of maleic acid, where the percentage increased from 95.65% to 99.01% when the concentration was increased from 0.01 to 0.075 M standard. It was also observed that the percentage of maleic acid extraction increases with increasing secondary amine concentration from 0.044 to 0.22 M. The percentage of acid increases with increasing temperature from 25 to 40 °C. The maleic acid extraction was more efficient in acidic medium, as it was noticed that the separation efficiency decreases as the acidity function increases in the neutral and basic medium.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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Preeclampsia symptoms and liver function tests in women with pre-eclampsia: comparison with a normal pregnant woman

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A B S T R A C T

Preeclampsia is a pregnancy-related condition that is linked to elevated blood pressure and proteinuria. It is one of the main causes of child and mother deaths in developed countries and only affects pregnant women during the second and third trimesters of pregnancy. Due to normal hepatic markers during pregnancy, the purpose of this study is to examine these factors in pregnant women and their association with disorders such as preeclampsia. Thus, the liver function tests in pre-eclampsia and normal pregnancy were compared. This study included 100 pregnant women after 20 weeks of pregnancy, divided into two groups, Group A is the preeclampsia group, which consisted of 60 preeclamptic women, whose blood pressure was greater than 140/90 mm Hg and whose proteinuria in a 24-hour period was greater than 300 mg. and Group B is the control group, which consisted of 40 pregnant women with normal blood pressure, The Obstetrics and Gynecology Department of the Teaching Governmental Hospital in the northeastern Libyan city of El-Baida provided the samples. In both groups, study assessed the serum activities of the liver enzymes ALT, and AST, and the level of total bilirubin. According to the findings, there was no discernible difference between the two groups' total bilirubin levels. The serum activities of ALT and AST across the two groups did, however, differ significantly ($p<0.05$). The results of this study indicate that preeclampsia-affected pregnant women had hepatic biomarkers that were higher than those of healthy pregnant women.

1 Introduction

In around 5%–10% of all pregnancies, hypertension during pregnancy raises the risk of complications (Cunningham *et al.*, 2010). It is third only to hemorrhage and embolism in terms of the primary causes of pregnancy-related mortality, and it causes severe maternal and perinatal morbidity (Mackay *et al.*, 2001 and Munazza *et al.*, 2011). According to Jeyabalan, 2013 and Panda & Mondal, 2018 preeclampsia (PE) is a syndrome that only affects pregnant women during the second and third trimesters of pregnancy and can impact different organ systems. It rarely occurs before 20 weeks of pregnancy, like in hydatidiform moles (Dutta, 1998 and Patil *et al.*, 2016).

Vasoconstriction and thickening of the vascular medium, that reduce vascular capacity and raise peripheral resistance, are the primary causes of preeclampsia (Munazza *et al.* 2011). Preeclampsia's exact aetiology is still not well understood. (Munazza *et al.*, 2011). Almost every organ is impacted. placenta, maternal immune response, vascular illness, genetic predisposition, and low calcium levels in the mother are some of the variables that seem to play a part. Preeclampsia has a placental origin, and placenta removal at birth is the first step in preeclampsia treatment. (Cnossen *et al.*, 2006 and Munazza *et al.*, 2011). The increase in peripheral vascular resistance in these ladies is probably what is causing their hypertension.

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PE 3 million preterm birth has unclear underlying pathophysiology, but it is currently thought that reduced placental perfusion—which results from shallow cytotrophoblast migration toward the uterine spiral arterioles and causes inappropriate vascular remodeling and a hypoperfused placenta is the primary initiating event in PE (Roberts & Gammill, 2005 and Amaral *et al*., 2015). As the pregnancy progresses, this placenta becomes ischemic, which causes the release of substances that promote maternal vascular endothelial dysfunction (Amaral *et al.*, 2015). One of the main phenotypes of preeclampsia, endothelial dysfunction causes broad vasoconstriction and decreased blood flow to numerous organs. Additionally, pre-existing illnesses like inadequate nutrition, diabetes, and obesity are risk factors for PE and may worsen the mother's reaction to substances released from an ischemic placenta (Brennan *et al.*, 2014).

While 800 women die from pregnancy complications around the world every day, 3 million preterm births reported each year are related to preeclampsia (Serrano *et al.*,2004, Kinney *et al*., 2012, and Amaral *et al.*, 2015). Other than an early birth of the fetus, there is no effective treatment for this pregnancy illness (Amaral *et al.*, 2015).

According to the working group categorization, PE is identified when a pregnant woman presents with proteinuria (i.e., urinary protein ≥300 mg/24 h or ≥1+ dipstick). and high blood pressure (BP), which is defined as BP ≥140/90 mm Hg. (Moser, 2001, Mammaro *et al*., 2009, Cunningham *et al.*, 2010, and Panda & Mondal, 2018). High blood pressure is linked to considerable organic dysfunction after 20 weeks of pregnancy, as well as a high maternal mortality rate due to complications like eclampsia, HELLP syndrome, and edema (Kedziora *et al*., 2021). Severe preeclampsia is defined by one or more of the following criteria: elevated blood pressure 160 mmHg systolic, or 110 mmHg diastolic, on two occasions at least 6 hours apart, on bed rest; with proteinuria ≥5 g in a 24-hour urine collection; and it is characterized by at least one of the following functional symptoms: headache, hyperreflexia, oliguria, epigastric or right upper quadrant pain, impaired liver function, and thrombocytopenia (HELLP syndrome). (Munazza *et al*., 2011).

Preeclampsia is further complicated by the involvement of the liver and kidney to varying degrees (Townsend *et al.*,2016 and Panda & Mondal, 2018). Liver involvement manifested as right upper quadrant abdominal pain or elevated transaminase levels, is one of the main clinical features of preeclampsia (Nachshon *et al.*, 2022). The liver is a vital organ in our bodies that performs numerous functions. The levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are normal. As a result, measuring these enzymes during pregnancy can be used to diagnose liver disease (Bacq, 2000-2013 and Panda & Mondal, 2018). Preeclampsia is the most common cause of liver function test (LFT) abnormalities, which occur in 3% of pregnancies (Angel, 2000, Munazza *et al*., 2011, and Das *et al*., 2013). The liver diseases peculiar to pregnancy have a characteristic time of onset. In the last trimester, liver disease with abnormal LFT, nausea and/or vomiting, and abdominal pain is caused by severe preeclampsia, HELLP syndrome, or acute fatty liver of pregnancy with or without sub-capsular hepatic haematomas, which overlap (Burroughs, 1998, and Munazza *et al*., 2011). Patients with HELLP syndrome are subsets of those with severe preeclampsia who are more likely to experience multiple system dysfunction (Weinstein,1982, Munazza *et al*., 2011, and Das *et al*., 2013). During Pre-eclampsia liver dysfunction has serious consequences. An increase in LFT results is observed in pre-eclampsia with HELLP syndrome.

ALT and AST levels may be elevated as well, and hyperbilirubinemia may occur, particularly in the presence of haemolysis. The lesion that causes elevated serum liver enzyme levels is most likely periportal hemorrhagic necrosis in the periphery of the liver lobule. Haemorrhage under the liver capsule can be so severe that the capsule ruptures, resulting in potentially fatal intraperitoneal bleeding (Simith *et al*., 1991, Burroughs, 1998, Munazza *et al.*, 2011, Das *et al*., 2013, and Lodhi & Roy, 2018). Analyses of hepatic function and integrity, such as AST, ALT, lactate dehydrogenase (LDH), bilirubin, albumin, and international normalized prothrombin time ratio, are frequently quantified in the routine clinical management of women with PE. Previous research has yielded conflicting results regarding the ability of LFT to predict adverse maternal outcomes. While some studies found strong associations between AST, ALT, LDH, and bilirubin levels and adverse outcomes, others found only weak or no associations (Magann *et al*., 1993, Sibai, 2004, Dadelszen *et al.*, 2004, Hupuczi *et al*., 2007, and Kozic *et al*., 2011). LDH reflects both hemolytic cell damage and hepatic dysfunction, bilirubin reflects both hemolysis and hepatic dysfunction, and AST reflects both tissue damage and hepatic dysfunction (Kozic *et al.*, 2011).

The current study was aimed to compare liver function parameters in pregnant women with preeclampsia and normal pregnant women.

2 Materials and Methods

A hundred pregnant women ranging in age from 17 to 45 years were admitted to the Obstetrics and Gynaecology Department of the Medical Center at Al-Bayda in Northeast Libya. Cases were sampled from

February 2019 to January 2020. The participants were split into two groups. Group A included 60 pre-eclampsia cases. After 20 weeks of gestation, Group B included 40 healthy pregnant women. Serum bilirubin levels as well as plasma levels of the liver enzymes ALT and AST were measured. A venous blood sample (5ml) was drawn at random from the pregnant women and placed in a sterile disposable syringe before being transferred to centrifuge tubes and allowed to clot for 30 minutes. The sample was centrifuged at 3000 rpm for 10 minutes to separate the serum, which was then stored at -20°C until analyzed. The serum was used to calculate alanine transaminase, aspartate transaminase, and bilirubin. The liver functions were determined using a Spectro-Photometer 4040V5+ chemical analyzer.

Statistical Analysis:

Minitab version 17 was used to analyze the data. The quantitative data were expressed as a mean ± SD, and percentage. The following statistics were performed: descriptive statistics and an independent samples t-test.

3 Results

Table (1) and Figures 1–4 show the liver function test parameters in the control and pre-eclampsia groups. The mean age in pre-eclampsia cases was 33.62, while it was 28.77 in controls. The mean AST value in pre-eclampsia cases was 22.53, while it was 15.63 in controls. The mean ALT level in pre-eclampsia patients was 24.08, while it was 16.00 in controls. The mean serum bilirubin level in pre-eclampsia patients was 0.550, while it was 0.533 in controls.

According to the current data, there was no significant difference in total bilirubin levels between the preeclampsia and control groups. However, at the end of the study, result found a significant difference in the ALT serum level between the preeclampsia and control groups ($p<0.05$), as well as a significant difference in the AST level between the two groups ($p<0.05$).

The mean value of headache symptoms in pre-eclampsia cases was 1.783, while it was 1.150 in controls. The mean value of visual symptoms in pre-eclampsia cases was 1.617, while it was 1.100 in controls. The mean value of chest pain in pre-eclampsia cases was 1.333, while it was 1.075 in controls. The mean value dyspnea in pre-eclampsia cases was 1.583, while it was 1.075 in controls. The mean value of nausea and vomiting in pre-eclampsia cases was 1.467, while it was 1.050 in controls. The mean value of epigastric pain in pre-eclampsia cases was 1.467, while it was 1.050 in controls. Table (2) depicts preeclampsia symptoms.

Among the 60 women diagnosed with preeclampsia, the number of women suffering from headache, vision disturbances, chest pain, dyspnea, nausea, vomiting and epigastric pain, respectively 47,37,20,35,28, and 28, and their percentages respectively 78.33, 61.67, 33.33, 58.33, 46.67, and 46.67, and the number of women who do not suffer from these symptoms, respectively 13,23,40,25,32, and 32, and their percentages, respectively 21.67, 38.33, 66.67, 41.67, 53.33, and 53.33. Table (3) demonstrates this.

The results of the correlation coefficient between the age of pre-eclamptic women and liver function parameters (LFT), and pre-eclampsia symptoms are presented in Table (4). Data found no link between the age of women with pre-eclampsia and their liver function disorder, as well as the symptoms associated with pre-eclampsia, implying that age plays no clear role in affecting the liver functions of women with pre-eclampsia and the symptoms associated with pre-eclampsia.

Result were used the box chart in this study because it is a convenient way of visually displaying the data distribution through their quartiles. The parallel lines extending from the boxes are known as "whiskers," and they are used to indicate variability outside the upper and lower quartiles. Outliers are sometimes plotted as individual dots that are in-line with whiskers.

Table (1). Comparison of liver function between control and pre-eclampsia groups

Parameter	Preeclampsia (60)	Control (n=40)	t-value	'p' - value
Age Mean ± SD	33.62 ± 6.50	28.77 ± 7.26	3.41	0.001*
AST (U/L) Mean ± SD	22.53 ± 9.97	15.63 ± 1.76	5.24	0.000*
ALT (U/L) Mean ± SD	24.08 ± 9.78	16.00 ± 4.28	5.64	0.000*
Serum Bilirubin (µmol/L) Mean ± SD	0.550 ± 0.362	0.533 ± 0.167	0.33	0.745

*Statistically significant: AST: Aspartate aminotransferase, ALT: Alanine aminotransferase.

Table (2). Comparison of symptoms between control and pre-eclampsia groups.

Parameters	Preeclampsia (n=60)	Control (n=40)	t-value	'p' - value
Headache Mean± SD	1.783± 0.415	1.150± 0.362	8.08	0.000*
visual symptoms Mean ± SD	1.617± 0.490	1.100 ±0.304	6.50	0.000*
Chest pain Mean ± SD	1.333± 0.475	1.075± 0.267	3.47	0.001*
Dyspnea Mean ± SD	1.583± 0.497	1.075± 0.267	6.62	0.000*
Nausea and vomiting Mean ± SD	1.467± 0.503	1.050± 0.221	5.65	0.000*
Epigastric pain Mean ± SD	1.467± 0.503	1.050± 0.221	5.65	0.000*

*Statistically significant

Table (3). Comparison between women with preeclampsia who have symptoms of preeclampsia and women who do not have them.

Symptoms	Preeclamptic women with symptoms		Preeclamptic women without symptoms	
	Number	Percentage %	Number	Percentage %
Headache	47	78.33	13	21.67
Visual symptoms	37	61.67	23	38.33
Chest pain	20	33.33	40	66.67
Dyspnea	35	58.33	25	41.67
Nausea and vomiting	28	46.67	32	53.33
Epigastric pain	28	46.67	32	53.33

Table (4). The correlation between age and liver function parameters (LFT) and symptoms of pre-eclampsia.

LFT and symptoms	r	P value
AST/GOT for pre-eclampsia	-0.124	0.344
ALT/GPT for pre-eclampsia	-0.035	0.790
Total Bilirubin for pre-eclampsia.	0.084	0.524
Headache	0.113	0.391
Visual symptoms	-0.107	0.414
Chest pain	0.051	0.698
Dyspnea	-0.018	0.892
Nausea/vomiting	0.105	0.425
Epigastric pain	0.219	0.093

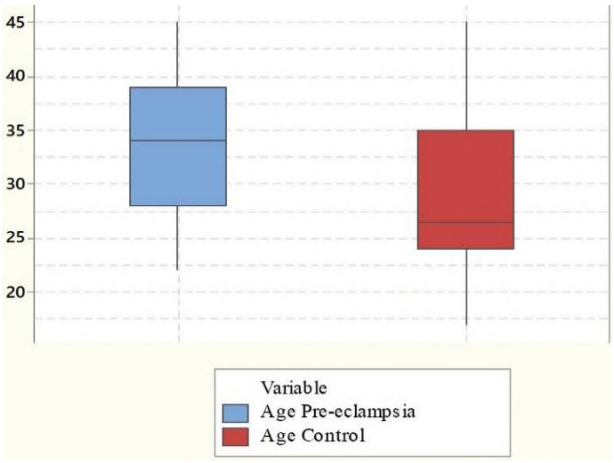

Figure (1). Boxplot for Age between pre-eclampsia and control groups

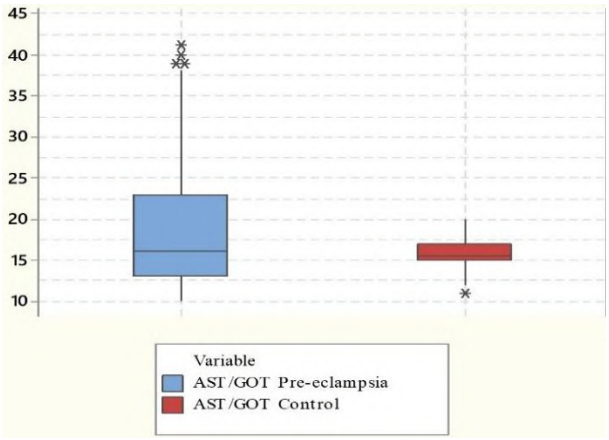

Figure (2). Boxplot for AST/GOT between pre-eclampsia and control groups.

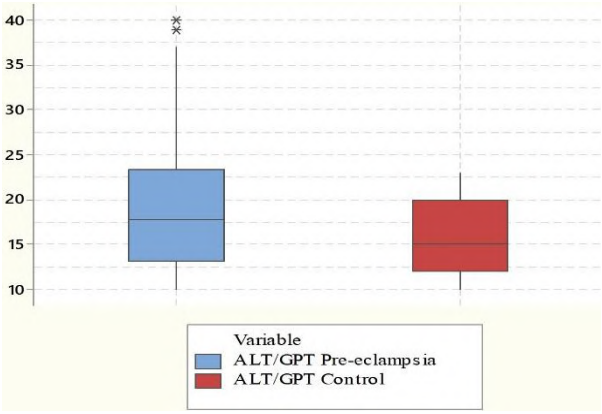

Figure (3). Boxplot for ALT/GPT between pre-eclampsia and control groups.

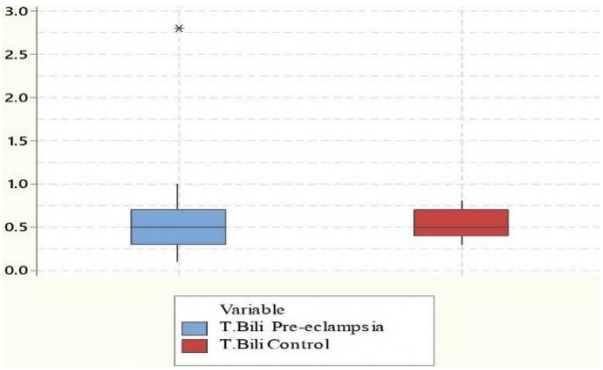

Figure (4). Boxplot for Total Bilirubin between pre-eclampsia and control groups.

4 Discussion

Although liver problems are uncommon in this disorder, severe preeclampsia is linked to perinatal illness and death. In fact, it is the most common cause of hepatic sensitivity and liver impairment during pregnancy, and 2%-12% of cases will develop HELLP syndrome, which manifests as the following three symptoms: hemolysis, elevated liver enzymes, and low platelet count.

Although liver involvement in preeclampsia does not necessitate treatment, it is an indicator to prevent more serious disorders such as eclampsia, hepatic rupture, or necrosis (Hay, 2008, and Hassanpour & Karami, 2018)**.** LFT are abnormal in 20% to 30% of pregnancies associated with preeclampsia (Borglin, 1958, Romero et al., 1989, and Hassanpour & Karami 2018) and are linked to poor maternal health and embryonic outcome. **(**Verhaeghe *et al*., 1991, Hay, 2008 and Hassanpour & Karami, 2018**).**

In the current study, LFT parameters such as ALT, AST, and total bilirubin in the two study groups were evaluated. The control group that consists of 40 healthy women with normal blood pressure and ages ranging between 17-45 years, in this group the levels of LFT parameters it was within normal limits, and the PE group, which included 60 women with PE, and their ages ranged between 22-45 years. (Patil *et al.* 2016).

In current study, observations wre found that there are statistically significant differences between the preeclampsia group and the control group in the level of ALT and AST enzyme, which was significantly elevated in the preeclampsia group when compared to the control group, and this is in line with the findings of Lodhi & Roy, 2018 in them study, where indicated that serum ALT and AST of pre-eclamptic women was significantly (P<0.001) elevated from their normotensive pregnant counterparts.

present results agrees with the results of (Malvino *et al*.,2005) who said that in pre-eclampsia the serum transaminase level was raised to >10 U/L and that of ALT to 271±297 U/L and Serum AST level in pre-eclampsia was also found more than 70 U/L which rose up to 209±178 U/L in eclampsia. Hassanpour & Karami (2018) showed that there was a significant difference in the serum ALT level between normotensive pregnant women and preeclampsia pregnant women (*p<0.05*). Ohotu *et al.* (2023) they recorded significant increase in the liver enzymes involving the ALT and AST and Alkaline Phosphatase (ALP) levels in the preeclamptic patients compared to the non-preeclamptic controls.

Khan *et al.* (2023) observed in their study that (ALT and AST) were significantly elevated among preeclamptic women (p<0.05). According to Munazza *et al*. (2011) and Patil *et al*. (2016), severe preeclamptic and eclamptic women had serum ALT levels that were significantly (*P<0.001*) higher than those of normotensive pregnant women, and preeclamptic cases had mean serum AST levels that were significantly (*P<0.001*) higher than the normotensive control group.

According to Dacaj *et al.* (2016), pregnant women with PE had statistically significant higher levels of AST, ALT, LDH, and total cholesterol than pregnant women in good health. When researching severe preeclampsia. Rath *et al*. (2000) found that the enzymes ALT and AST activities were both increased. In preeclamptic gestation, Makoyana *et al*. (2002) also noted elevation in AST and ALP activities.

Based on a study by Panda & Mondal, (2018), whose goal was to compare the results of liver and kidney function tests among preeclampsia patients between the ages of <35 and ≥35 years, in contrast to our finding, they found that the mean serum ALT levels in both the control and study groups were within the normal range. However, the mean AST and mean ALP levels revealed elevated values in both groups, which is consistent with our finding regarding AST. Makuyana *et al*. (2002) disagreed with current result at a compared liver function in normal and preeclamptic gestation, that did not notice differences in the level of ALT.

The influence of hypoxia on the liver during preeclamptic pregnancy accounts for the elevated serum level of AST in PE. Endothelium disruption causes prostacyclin levels to drop and thromboxane levels to rise. The ratio of PgI2/TxA2 is increased in favor of thromboxane, causing the liver's blood arteries to constrict. The consequences of hypoxia on the liver will result in hepatocyte necrosis and degeneration, which will raise AST levels. PE results in the release of several mediators (fibronectin, thrombomodulin, endothelin-l, and thromboxane) from the liver and blood vessel endothelium, which results in vasoconstriction and hepatic hypoxia, hypoxia raises the level of ALT

According to (Cines *et al*.,1998, Dacaj *et al*., 2016, Patil *et al*., 2016 and Lodhi & Roy, 2018).

Shukla *et al*. (1978) and Hassanpour & Karami, (2018) mentioned that in normal gestation, ALT and AST are lower than in non-pregnant age-matched women, however, AST changes to a lesser amount. Mcmahon *et al*. (1993) and Hassanpour & Karami, (2018) pointed out that the primary fluctuations in liver function evaluation may be due to red cell demolition and ultimately happens liver damage. showed their results that liver damage in pregnant women with PE, and other biomarkers were higher in pregnant women with PE compared with normal pregnant women.

In this investigation, there were no statistically significant differences between the control group and the preeclampsia group in terms of T.B. levels. These findings are in line with those of Makuyana *et al*. (2002), who observed no differences in serum bilirubin when they evaluated liver function in normal and preeclamptic gestation. Patil *et al*. (2016) found that liver function parameters were significantly elevated in the severe preeclampsia and eclampsia group except for serum bilirubin level, which is not significantly higher when compared with the control group of the same age.

On other hand, present findings differ from those of Lodhi & Roy, (2018) who found that the concentration of serum bilirubin was considerably greater ($P<0.001$) in pre-eclampsia patients prior to delivery than in the control group of same-age and parity individuals with normal blood pressure, and also differs from the results of Malvino *et al.* (2005) who indicated that in HELLP syndrome serum bilirubin concentration was elevated from its normal value to about >1.2 mg/dl. Similar findings were made by Jaleel *et al.* (1999) and Munazza *et al*. (2011), showing pre-eclamptic women had significantly higher serum bilirubin levels than normotensive pregnant women. Hassanpour & Karami, (2018) also found that although direct bilirubin levels were not significantly different from the normal group, total bilirubin levels were higher in pregnant women with PE.

Various investigations have been examined the evaluation of liver function tests and liver damage in pregnant women with preeclampsia and normal pregnant women so that obtained different results. For example; a study by Girling *et al.* (1997) stated that the rate of liver function tests is less in normal gestation than the scope of reference presently used.

Girling *et al*, (1997) and Hassanpour & Karami, (2018) reported that AST, ALT, bilirubin, and GGT were each lower in uncomplicated pregnancy than in the non-pregnant laboratory reference ranges. In the pre-eclampsia group, 37% of the cases with higher liver function tests were abnormal only by the new reference ranges.

5 Conclusions

A frequent complication of pre-eclampsia is liver damage. While the amount of total bilirubin remained unaffected, we observed a disruption in some liver function indicators, where there was an increase in the activities of alanine transaminase and aspartate transaminase, which can be taken into account when predicting preeclampsia. To support the current theory, we propose additional research.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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Evaluation of Efficiency of Two Local Rhizobium Leguminosarum Isolates on Root Nodulation and Growth of Faba Bean (*Vicia faba L.*)

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A B S T R A C T

This study was conducted at the Libyan Biotechnology Research Center to investigate and evaluate the efficiency of two local isolates of Rhizobium Leguminosarum bacteria in root nodulation and improvement of the growth of faba bean. The two studied isolates were isolated from two geographically different regions in Libya (Qasir Ibn Ghashir and Shahhat). The experiment was designed according to the completely randomized Design (CRD). The experiment included two treatments (isolates) and three replicants of every isolate. The results showed superiority of R2 isolate in all studied growth characteristics (plant height, number of leaves, root length, root fresh weight, and the fresh and dry weight of stems and leaves) when compared to R1 isolate. Treating the plant with R2 isolate increased also the number of active nodules and the total weight of nodules. The results of all examined characteristics indicate that R2 isolate which is isolated from shahhat is significantly better in improving plant growth, which is as a result of the effect on nitrogen fixation and production of some plant hormones.

1 Introduction

Faba Bean (*Vicia faba L.*) is considered one of the oldest crops cultivated all over the world (Mínguez and Rubiales, 2021). The Mediterranean countries, Ethiopia, Egypt, China, Afghanistan, India, Northern Europe, and North Africa are the major producers of faba beans (Rahate *et al*,. 2020). Among the more than 50 bean producing countries, about 90% of production is in Asia, the European Union and the African region (FAO, 2020).

Vicia faba is one of the winter crops belonging to the legume family, whose beans contain high percentage of protein, about 25 - 40% (Natalia *et al*., 2008). In addition, the beans contain carbohydrates that may zreach 56% in most varieties, and this increases the importance of the crop due to its high nutritional value. In addition, one of the faba bean cultivation benefits is the improvement of soil properties, through the symbiotically fixation of atmospheric nitrogen by Rhizobium (Zaki *et al*., 1997), therefore, Faba bean plants are used in agricultural rotations for improving soil properties (Carmen *et al*., 2005). Faba Bean is adapted to a wide range of soil pH 4.5-8.3, which resulted in yield increasing. Dry beans are also used as an animal fodders to improve the productivity. Faba bean is an important crop in regard to the environmental, nutritional and economic point of view (Xiao *et al*., 2021).

Conversely, Libyan soils are inefficient in nitrogen. Bio-fixed nitrogen through the symbiotic relationship between legumes and Rhizobium as a source of nitrogen supply is of great importance (Martin,1982), and therefore Rhizobium plays a major role in agriculture by providing soils with a part of the nitrogen fixed from the atmosphere (Verma and long ,1983). The biological

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fixation of atmospheric nitrogen represented by the symbiotic relationship between Rhizobium and leguminous plants requires sufficient Rhizobium number in the soil. Treating the legume seeds with a specialized Rhizobium species before sowing has economic benefits, as it is considered an effective way to increase soil fertility (Hardarson, 1993).

In a field study, the results showed there was a significant effect of inoculation with root nodulation bacteria, which led to proportion increasingof protein, carbohydrates, and fiber in seeds (Abbas and Majid ,2006). Testing of 12 Rhizobium strains isolated from Tigre highlands in northern Ethiopia showed that there were significant differences in the number of bacterial nodules, fresh weight, plant length, and nodule color variation (Alemayehu, 2009). Turki, 2011 also found that inoculated faba bean plants by Rhizobium were better than un-inoculated ones in regards to nodule numbers, nodule weights, shoot, nitrogen concentration, number of branches, shoot dry weight. Inoculation faba bean with rhizobium increased significantly the yield, seed moisture, ash, raw fibers and raw protein (Rugheim *et al*., 2012). It was found by Osama et al., 2021 that inoculation by Rhizobium led to a significant increase in nodule numbers, shoot and root growth, and the nitrogen content in plant and soil.

The aim of this study was to obtain Rhizobium strains that are highly efficient in nitrogen fixation to use them as a bio-fertilizer which is an alternative to mineral nitrogen fertilizers that has negative effects one human and environment.

2 Materials and Methods

This study was conducted at the Libyan Biotechnology Research Center using faba bean plants and the bacterial species Rhizobium Leguminosarum.

2.1 Bacteria Used in the Study

Two isolates of the species Rhizobium Leguminosarum were isolated from two geographically different regions of Libya (Qasir Ibn Ghashir and Shahhat) at the Libyan Biotechnology Research Center according to the known isolation procedures used to get pure isolates by using the medium of yeast extract mannitol agar (table 01). The isolates were observed in a fridge in agar slant tubes until using them in the field experiment.

Table 1: Composition of yeast extract mannitol agar

Parameters	Value
Yeast Extract	0.5g
Mannitol	10g
$MgSO_4.7H_2O$	0.2g
K_2HPO_4	0.5g
NaOH	0.1g
Agar	15g

Bacterial inoculums of the two isolates (R1: Qasir Ibn Ghashir isolate, R2: Shahhat isolate) were prepared in a yeast extract mannitol solution. The used tools, distilled water and the medium were sterilized by Autoclave AUX-512-020D by 120C° and a pressure of 15 psi for 20 minutes (Zaki, 1997). The inoculated media were incubated using a shaker incubator (Shaking Incubator 3023-3033 GFL) by 28C° for 72 hours.

2.2 Characteristics of Used Soil and Irrigation Water

The soil properties mentioned in tab. 02 were determined according to the known procedures of soil analysis. Calcium carbonate was estimated by calciminer. The Jackson method, 1973 was used to estimate the available phosphorus in the soil. The total soil nitrogen was estimated according to Kjeldahl method, while the zinc concentration was according to DTPA method. For estimation of available potassium was used a flam photometer.

Table 2: Some properties of used soil

parameters	Value
Physical properties	
Sand	77.14%
Silt	8.18%
clay	14.68%
Soil texture	sandy loamy
Chemical properties	
Organic matter	0.33%
Calcium carbonate	5.0%
Available zinc	0.26 ppm
Total nitrogen	0.25%
available phosphorous	0.6 ppm
available potassium	97 ppm
pH	7.95
EC	1.04 dS/m

Irrigation water samples were taken in a sterile bottles to determine the mentioned parameters in table3.

Table 3: Chemical properties of irrigation water

parameters	Value
pH	6.8
EC	1.85 dS/m
TDS	1184 ppm
K^+	39.6 ppm
Na^+	105 ppm
Ca^{+2}	58 ppm
NO_3	10.81 ppm

2.3 Seed Inoculation and Planting

Faba beans of the cultivar Nbella Mora obtained from an agricultural store were sterilized using a sodium hypochlorite solution and ethanol 95%. Then, the seeds were treated with the isolated Rhizobium isolates after by soaking them in a solution of adhesive substance (sugar).

The inoculated beans were planted in polyethylene bags filled with 5 kg of a sieved soil. The experiment was designed according to the completely randomized Design (CRD). Two seeds were sowed in every bag of three bags (replicants) for every isolate (treatments R1 and R2). The soil was fertilized according to the crop requirements.

The plants were harvested after 49 days of sowing, the, the roots were separated from the shoots. comparison tests related to the vegetative growth, root growth and nodulation were done to compare between the two treatments.

Statistical Analysis

The statistical analysis were performed according to the Snedecor and Cochran method 1980. For the comparison of averages between treatments, the method of least significant difference (LSD) at 0.05 significant level using the SAS program 1999 were used.

2.4 Results and Discussion

2.4.1 Effect of Rhizobium Isolate on the Shoot and Root System

The results mentioned in Table 4 indicate that the two bacterial isolates are different in effect when compared the results of shoot and root system. According to these results, it's clear that R2 isolate is significantly superior in plant length, by which the plants were 7.06 cm taller than the plants treated with isolate R1, Also the root length, number of leaves and the fresh weight of the root system increased significantly in R2 treatment, by which the values were higher by 7.21 cm in the root length, 4.43 in the number of leaves and by 3.10 g in the fresh weight of the root system. Such increase in the studied attributes were also mentioned by Al-Tamimi 1998, Al-Baldawi 2004 and Saad 2011, which indicates that the strain is an affective factor in nitrogen fixation that enhances the plant nutrition.

The reason beyond the enhanced growth of the root system by R2 Isolate can be explained as a result of production of some plant metabolites such as Riboflavin, Cytokinine and Gibberellin which play an important role in root growth, and that improves the plant growth due to the increased uptake of nutritional elements (Dakora, 2003). R2 isolate as shown in table 05 lead also to a significant improvement in both wet and dry weights of both stems and leaves. That corresponds with the results obtained by Alemayehn, 2009 and Rugheim *et al*., 2012 in their studies of the effect of different Rhizobium strains on nitrogen fixation. The increased weight of the plants is an indicator of increased nitrogen fixation ratios (Somasegaran and Hoben, 1994). As nitrogen is the most important element in plant nutrition, this explains that these differences between the both isolates are due to different efficiencies of nitrogen fixation, by which the atmospheric nitrogen is converted into available form to the plant which is used to create amino acids and proteins that lead to improvement of the plant growth.

Table 4: Effect of Rhizobium Isolate on the shoot and root system

Treatment	Plant height (cm)	Leave number	Root height (cm)	root Fresh weight (g)
R1	52.14b	23.97b	11.47 b	17.33 b
R2	59.20a	31.18a	15.9 a	20.43 a
LSD 0.05	3.17	2.29	0.25	0.06

Table 5: Effect of Rhizobium Isolate on the fresh and dry weights

Treatment	Stem Fresh weight (g)	leaf Fresh weight (g)	Stem dry weight (g)	leaf dry weight (g)
R1	12.79 b	6.92 b	1.14 b	1.85 b
R2	14.41 a	8.63 a	1.35 a	2.05 a
LSD 0.05	0.01	0	0.008	0.01

2.4.2 Effect of Rhizobium Isolate on the Nodulation of Faba Bean

The results mentioned in table 6 showed significant increasing in the number of effective nodules and also the total number and weight of nodules produced by the R2 isolate. Such increasing in weight and numbers corresponds with the results obtained by Alemayehn, 2009 and Saad, 2011. This explains that the increasing in nitrogen fixation by R2 isolate mentioned previously is related to the better activity of R2 isolate in root penetration, nodule formation and to the difference in reproduction and generation time between the two studied isolates.

Table 6: Effect of Rhizobium Isolate on the nodulation of fava bean.

Treatment	Total number of nodules	Number of active nodules	Ratio of effective nodules %	Nodule total weight (mg/plan)
R1	28.4 b	54.3 b	65.61 b	85.73 b
R2	34.2 a	66.8 a	66.10 a	133.68 a
LSD 0.05	0.14	0.56	0.41	0.70

3 Conclusion

This study showed significant results in all studied attributes, which indicates that the Rhizobium strain is an affective factor in nitrogen fixation, and as a result of this, R2 isolate which is isolated from Shahhat in eastern part of Libya was significantly more efficient in nitrogen fixation than the one isolated from Qasir Ibn Ghashir. That was shown as an enhancement of shoot and root system which is related to increasing in attributes connected to the nodule formation (nodule number, nodule weight and number of effective nodules). The different behavior of the two isolates can be explained as a result of an adaptation to the different environments.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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In vitro Micropropagation of Ginger plant (*Zingiber officinale*)

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The study was conducted for the purpose of micro propagation of ginger plant (Zingiber officinale) through use of plant tissue culture technique to identify the best plant micro propagation conditions. The plant samples were sterilized superficially by immersing them in the Clorox solution, and then the sterilized plants were cultured in MS media in order to obtain free contamination culture. After the sufficient number of ginger plants had obtained the plants were replanted in MS media supplemented with several concentrations of (BA and NAA) for the purpose of obtaining the best vegetative growths. The treatments were divided into four treatments as T1 (control treatment contains MS without hormones), T2 (MS +0.1mg/l NAA+1mg/l BA), T3 (MS+0.1mg/l NAA+2mg/l BA) and T4 (MS+ 1mg/l NAA). The results of this study showed that addition of 0.1mgNAA with 1 or 2mg/l BA improve the vegetative growth of ginger plant. It is also proved that the average of plant length, number of brunches and number of leaves was significantly higher in T2 and T3 compared with other two treatments. Furthermore, results proved that combination between NAA and BA gave the best vegetative growth. For the root site, the results showed that the culture media (MS) plus 1 mg / L of the growth regulator (NAA) resulted in the highest of root growth. A cording to our results addition 0.1mg NAA with 1 or 2mg/l BA would be the best choice for In vitro Micropropagation of ginger plan.

1 Introduction

Recently medicinal plants are receiving a high attention throughout the world due to that people are returned to the natural for their diseases treatments whereas, the high demand on medicinal plants nowadays led to decrease in numbers of medicinal plants on the planet. In general, medicinal plants consider as a rich resources of ingredients that can be used in drug development. Thereby obtaining high amount of medicinal plant is became as the main aim for pharmacology sector due to its important role in drugs industries. In fact, medicinal and aromatic plants mainly propagated by normal vegetative propagation methods. However, the propagation method of medicinal plant should be improved and new technologies are needed in order to obtain more quantities every year to face the high demand on these plants in the globule market. Particularly the traditional methods of propagation face some challenges, which make these methods more difficult to apply. Therefore, prevention of contamination from different source of bacteria and fungi is necessary for successful culture of medicinal plants by using in vitro propagation (Dadi *et al*., 2020) also, in vitro Micropropagation is a method useful for the multiplication of selected genotypes and chemo types of some medicinal and aromatic plant species (Rout *et al*., 2001). In vitro propagation methods are essential components of plant genetic resource management and become extremely important for the conservation of rare

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pants (Sidhu, 2011). Generally In vitro propagation of important plants can offer considerable benefits, including rapid cultivation of species that have limited reproductive capacity and exist in threatened habitats (Ziba *et al*., 2016).Micropropagation is the process of vegetative growth and multiplication from viable and regenerative cells in various plant tissue culture techniques (Zhou and Wu, 2006). Moreover, in vitro propagation large numbers of identical plants can be produced within a limited space and time, which can be used as planting materials this technique is being used in the propagation of different medicinal plants (Dadi *et al*., 2020). Storage facilities maybe established at any geographical location and cultures are not subject to environmental disturbance such as temperature fluctuation, cyclones, insect, pests, and pathogen (Sharma and Malik, 2014). Application of the tissue culture protocols will facilitate research in to the enhanced production of antitumor compounds through different biotechnological strategies, such as plant cell, tissue and organ cultures, and large – scale cultivation in bioreactors. In vitro plant regeneration depends on the type of growth regulators used and explants source while, in vitro cultured explants respond differently according to the growth regulators added to the cultured medium, thus contributing to the germplasm conservation of this endangered and valuable medicinal species in the wild (Ziba *et al*., 2016). Recently large numbers of medicinal plants are being harvested from their wild habitat and due to this over harvesting several species have become extinct plants (Kumari and Priya, 2020). Ginger (Zingiber officinale Roscoe) is belongs to the family of Zingiberaceae. This plant is an important tropical horticultural plant, valued throughout the countries as an important spice for its nutritional and medicinal properties (Devina *et al*., 2016). This plant is an important tropical horticultural plant, value throughout the countries as an important spice for its nutritional and medicinal properties. Ginger is vegetative propagated through use of rhizome sand mainly cultivated in many countries including India, China, Japan, Indonesia, Australia, Nigeria and West Indies (Ravindran and Nirmal, 2005). The plant has been used as medicinal plant to treat cancer diseases (Zang *et al*., 2021). Ginger is a perennial herb and commercially grown in many tropical regions and is a native to tropical south east of Asia (Serasan and Sileshi, 2011). This plant is an unfertile species that failed to set seed. Ginger is a herbaceous perennial grown as an annual crop. These are plants of tropical and subtropical regions distributed mainly in East Asia. Several authors have quoted different figures for the total number of genera and species but it is probably appropriate to quote the world record at least51 genera and 1500 species (Newman, 2001). Ginger has many fibrous roots, pseudo stem with leaves, and an underground stem rhizome. It is one of the top 20selling herbal supplements in the United States (Gang and Ma, 2008). The rhizome is the tissue used for a lot of purposes, such as vegetative propagation and the storage of food materials (Ravindran and Nirmal Babu, 2005). Ginger is vegetatively propagated through underground rhizomes and mainly cultivated in many countries including India, China, Japan, Indonesia, Australia, Nigeria and West Indies (Ravindran and Nirmal, 2005). Propagation of zingier using traditional methods are facing some problems such as appearance of plant disease, luck of rhizomes, limited to a specific season of the year and conservation of zingier available all the year. However, collection may occur at any time independent of flowering period for each species (this assumes that seed material is not required) (Nada opal *et al*., 2011) while, there is the potential of virus elimination from contaminated tissue through meristem culture (Behera *et al*., 2010). Thereby In vitro propagation has long been recognized as an efficient means for rapid clonal multiplication and conservation. In fact in vitro culture became as the best method as a continuous source of supply of disease free planting material for commercial utilization. (Kambaska and Santilata, 2009). Du to its anti-inflammatory properties, ginger is used as a medicinal plant to treat a wide area of illnesses and disorders, such as arthritis, in flammatory bowel disease, cancer, Alzheimer's disease and the common cold. Moreover, this plant success fully used as anti-allergic, antiemetic, anti-hepatotoxic, anti-flammatory, anti-nauseate, antiseptic, antitussive, cardio vascular, digestive, and hypoglycemic activities (Duke et al., 2003). The plant cannot be sexually propagated duo to that the rhizomes are used for its vegetative propagation (Nair, 2019). Moreover, the rhizome of this plant considers as an economical part and using large quantities of rhizomes as starting material for propagation negatively affects its supply in the globule market (Nisaretal, 2021). Ginger is reproductive and is only propagated vegetative (Ravindran and Nirmal Babu, 2005). On the other hand, ginger plant affected by several diseases which may act the propagation of this plant and then decrease the number of plant every year, rhizome rot caused by Pythium spp. And Ralstonia solanacearu mare major diseases affecting ginger.

The utility, the various method of propagation includes efficient cost, effective method of in vitro multiplication is essential for improvement of ginger. However, plant tissue culture technology exists as one of the most important methods being used to obtain moor plants and avoid the problems which may face with vegetative propagation. Although several studies have proved the successfully use of tissue culture techniques propagation method of ginger (Serasan and Sileshi, 2011), it is very important to develop an effective protocol because the efficiency of tissue culture is dependent on several factors, such as plant growth regulators, physiological state of the explants, etc. The plant cannot be sexually propagated duo to that its rhizomes are used for its vegetative propagation (Nair, 2019). Moreover, the rhizome of this plant considers as an economical part and using large quantities of rhizomes as starting material for propagation negatively affects its supply in the globule market (Nisar *et al*., 2021). Ginger is reproductive and is only propagated vegetative (Ravindran and Nirmal Babu, 2005). On the other hand, ginger plant affected by several diseases which may act the propagation of this plant and then decrease the number of planted plants every year, rhizome rot caused by Pythium spp. And Ralstonia solanace arum are major diseases affecting ginger. The utility, the various method of propagation includes efficient cost, effective method of in vitro multiplication is essential for improvement of ginger. However, plant tissue culture technology exists as one of the most important methods being used to obtain moor plants and avoid the problems which may face with vegetative propagation. Although several studies have proved the successfully use of tissue culture techniques propagation method of ginger (Serasan and Sileshi, 2011), it is very important to develop an effective protocol because the efficiency of tissue culture is dependent on several factors, such as plant growth regulators, physiological state of the explants, etc.

The present study was carried out to develop an effective protocol for in vitro propagation and plant regeneration of ginger. Thereby plant tissue culture technology came as alternative method for propagation of some medicinal plants.

2 Materials and Methods

This study was conducted in the laboratory of plant tissue culture entered the Libyan Center for biotechnology research located in Tripoli Libya.

Explants preparation

Fresh ginger rhizomes were brought from the local market. Directly ginger rhizomes were placed in a dark Place away from any light source in order to induce the plant to produce buds. After 30 to 40 days, the grown buds were taken for next stage.

Media preparation

The culture media is mainly used to provide the necessary plant needs of the macro and micronutrients beside source of carbon, vitamins and growth regulators needed for the study, whereas MS media has been selected and used to obtain an efficiency protocol forging Micropropagation.

Establishment of contamination-free tissue culture

This stage conducted for the purpose of obtaining clean MS media free of contaminations for the development of single nodes of ginger plant, whereas this stage started with preparation of Half basal of Murashige and Skoog 1962 (MS) salts nutrient medium with vitamins and supplemented with30g L-1 sucrose was used for in vitro seedlings germination. All cultures pH was adjusted to 5.8 with 1N KOH or 1N HCl, then with 7g L-1 agar prior to autoclaving at 121ºC and 1.2kg cm-2 for 15 minutes. Directly jars contained culture media transferred and put in an autoclave at a temperature of 121°C and an atmospheric pressure of 1.02 bar for 15 minutes for the sterilization stage.

Table (1) MS media preparation

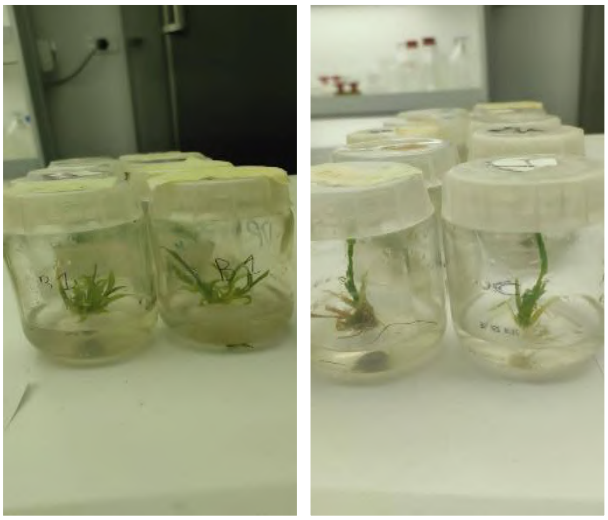
Macro elements		**Micro elements**	
Salts	mg/l	Salts	mg/l
$NH_4. NO_3$	1650	H_3BO_3	6.2
KNO_3	1900	$MnSO_4. H_2O$	16.9
$CaCl_2.2H_2O$	440	$Zn\ SO_4.7H_2O$	8.6
$Mg\ SO_4.7H_2O$	370	KI	0.83
$KH_2\ PO_4$	170	$NaMOO_4.2H_2O$	0.25
Vitamins		$CUSO_4\ .5H_2O$	0.025
Myo-inositol	100	$CO\ CL_2.6H_2O$	0.025
Nicotinic acid	0.5	NaEDTA. $2H_2O$	37.3
Thiamine. HCl	0.1	$FeSO_4. 7H_2O$	27.8
Pyridoxine. HCL	0.5		
Agar	7000		
Sucrose	30000		

Surface Sterilization of Explants

After growing buds reaching length of 1-2 cm the grown buds were collected from giber rhizomes and prepared for surface sterilization stage. The collected buds were cut into parts of suitable lengths then placed under running water for 30minutes to remove surface contaminants from soil and insect residues ,afterwards samples were transferred to the laminar airflow cabinet for sterilization by using ethanol with 70% concentration for two minutes then ginger buds sterilized by sodium hypochlorite (Clorox) with concentrations 2.5% and for 20 minutes with keeping stirring of the samples time to time for sterilization from bacteria and fungus. finally, samples were washed by using sterilized distill water for three times with five minutes each time to remove the toxic effect of sodium hypochlorite solution (Aazami et al.,2010).

Culture stage

This stage started by planted the selected and sterilized buds in containers supplemented with MS media, whereas 2-3 buds placed in each container for the purpose of obtaining tissue cultures free of contamination, afterwards samples were transferred to growth room and incubated at 16 hour light /day and 8 hour dark/day the light intensity was (1-s1Mm 30 µ) by using fluorescent lamps , temperature 25±2C◦ and 40% humidity, while all the conditions were under control and the samples incubated for four to six weeks to be ready for use in next stage of the study



Figure(1): regenerated shoot lets of ginger plant after 45 days of culture.

Subculture Stage

After the whole plants being obtained through tissue culture technique the subculture stage started by transfer the obtained plants to cabinet room for subculture operations for the purpose of evaluate the effect of plant growth regulators on ginger propagated plants which already obtained from the previous stage figure (2). The plants were re-planted in MS media supplemented with different concentrations of plant regulators as follows:
First treatment (T1) control treatment MS media without added hormones
Second treatment (T2) MS media + 1mg/l BA + 0.1mg/l NAA
Third treatment (T3) MS media + 2mg/l BA + 0.1mg/l NAA
Fourth treatment (T4) MS media + 1mg/l NAA

While two three plants were planted in each jar then all plant samples incubated in the growth room under the seam conditions which written above then after 45 days the results were taken and recorded.

Figure (2): plants incubated in growth room.

3 Results

This study conducted to determine the best protocol which can be successfully used for In vitro propagation of ginger plant. Thereby carefully, assessments must be done to obtain the best method. However, several studies were conducted in order to obtain satiable method for Micropropagation of ginger plant and medicinal plants in general. In fact, the efficiency of used method in Micropropagation depend on the ability of used culture media which usually contain a combination between oxen and cytokines to improve and enhance the plant growth as hole through parameters such as plant height, number of leaves and roots which

has been evaluated in this study. However, the result of this study were as follow.

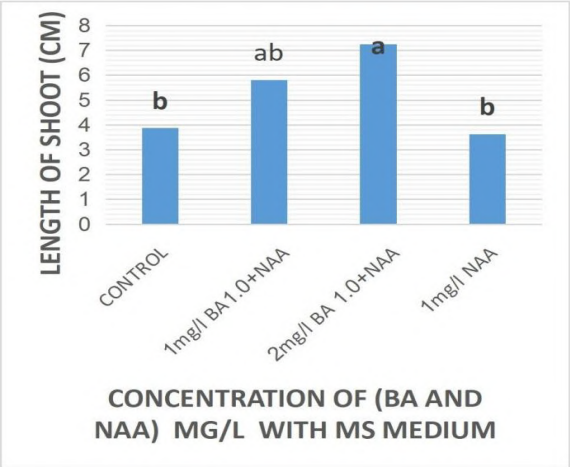


Figure (3): effect of plant growth regulators on plant length of ginger.

Plant Length Parameter

The results of this study figure (2) showed that the treatments T2 and T3 which contain a combination between NAA and BA , whereas (T2) contains MS media + 1mg/l BA + 0.1mg/l NAA and (T3) contains MS media + 2mg/l BA + 0.1mg/l NAA gave the best results for plant length this obtained results proved that use of MS media with a combination between NAA and BA led to obtain plants with satiable growth but the concentration of added NAA and BA must be selected carefully and tested, whereas in this study 0.1 NAA, 1 and 2 mg/l were added this results proved the positive role of NAA and BA growth regulators on plant growth improvement. Furthermore, plant length obtained from treatment of T2 and T3 was significantly higher than the other treatments which are.

T4 and control treatment, whereas the plants were grown in T3 reached a length of 7.2cm and plants grown in T2 reached plant length of 5.9 cm but no significant differences were found among those treatments.

This obtained results proved the positive effect of NAA and BA when added with satiable concentrations also addition of NAA alone to MS media was not able to improve plant growth and the same was found in control treatment. Our results were in the same line with Kheiry et al. (2018) who found that addition of BA and NAA improves ginger growth under use of plant tissue culture technology.

Number of Leaves

For number of leaves parameter figure (4) almost the same results as in plant length have been found. The results of this study showed that the treatments of T2 and T3 gave the highest number of leaves, which indicate the positive effect of NAA and BA hormones. This results proved that addition of NAA and BA to MS media increases the number of leaves formed by the cultured plant this also explains

that BA growth regulator induce the plant to produce more leaves which led to improvement in plant growth in general. The results of this study showed also that number of leaves produced in T2 and T3 was significantly than number of leaves produced in T4 and T1 (control treatment). While T2 and T3 reached an average of 28 and 31 leaves respectively, whereas no significantly differences were found between T2 and T3.

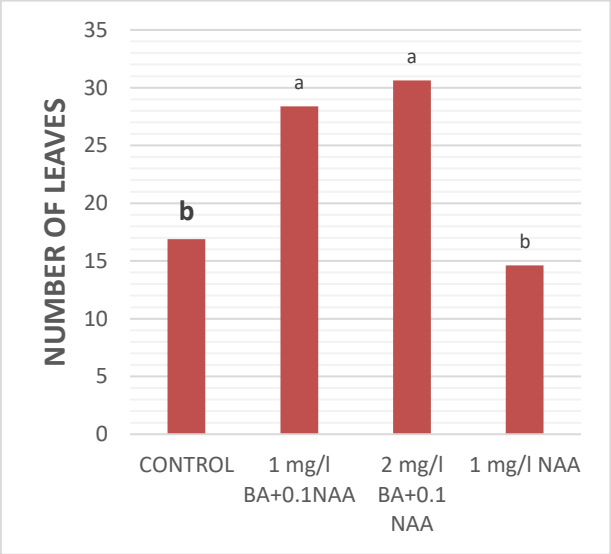


Figure (4): effect of plant growth regulators on number of leaves

The results showed also that T1 and T4 were not efficient compared with T2 and T3 which explain the positive role of plant growth regulators BA. Furthermore, according to our obtained results we found that the addition of 2mg/l BA increased the number of leaves and plant length which proves that this concentration is extremely satiable for ginger Micropropagation this has been proved by several studies. On other hand NAA when added to MS media without addition of BA as in T4 was not efficient and the number of leaves was low.

Number of Brunch's Parameter

Plant tissue culture technology has a major impact on both agriculture and industry sector by providing a large number of plants in short period of time to demand also it has contributed greatly to the advancement of agriculture science in recent times and it is an indispensable tool in modern agriculture nowadays (Garica *et al*., 2010). However, number of brunches increase the number of obtained plant through plant tissue culture and several hormones are used to increase number of brunches production. Our results figure (5) proved that addition of BA increases the number of brunches. The results showed that treatment of T2 (MS+0.5 mg/l NAA and1mg/l BA) and T3 (MS+ 0.5mg/l NAA and1mg/2 BA) gave the highest number of brunches. The results showed that the treatment 0.5 NAA+ 2mg BA were significantly higher than the rest of the treatments, especially the control treatment (MS), which demonstrated the positive effect of growth regulators on the growth of the ginger plant, and demonstrates the extent of the success of ginger plant propagation using cultivation technology.

Plant tissue and obtaining appropriate lengths for the cultivated plants at a specific time in preparation for transferring them to the replanting stage and the acclimatization stage which, succeeded in growing a plant that grows using the technique of vegetative cultivation (Villanor, c. c, 2010). The average of number of brunches in those treatments was significantly higher than other two treatments, which areT1and T4. While T2 and T3 produced, number of brunches reached to7and 6.2 brunches respectively and no significantly, differences were found betweenT2 and T3while, the lowest number of brunches were obtained inT1and T4. These results proved that T1and T4 were not satiable for ginger Micropropagation. However, no significant differences were found among T1and T4. The present results were in complete agreement with those indicated in studies of Khiry *et al*., (2020) that use of 2mg/l BA in combination with NAA improved the vegetative growth of ginger plant. The researcher reported also that present a satiable response of zingier plant to Micropropagation by tissue culture technology in all the treatments even control treatment moreover the results showed that the treatment of 2mg/l BA gave the highest average of obtained number of brunches and root system growth. necessitates This results proved that application of tissue culture techniques as a solution to these problems (Nayak and Naik, 2006).

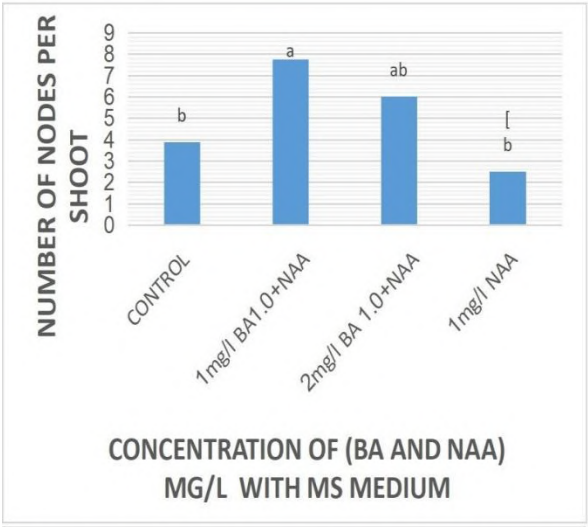


Figure (5) effect of plant growth regulators on number of brunches.

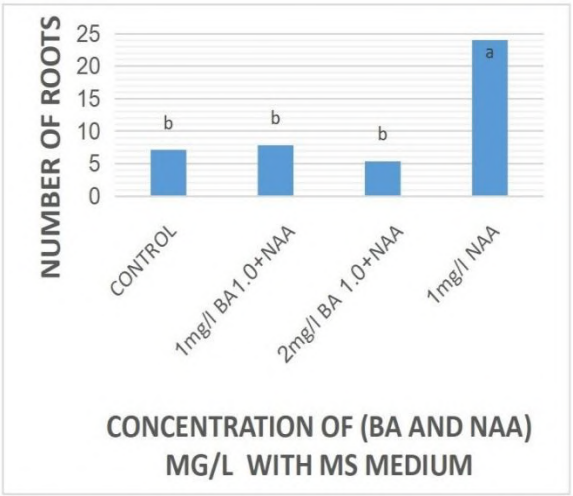


Figure (6) Effect of plant growth regulators on number of roots

4 Discussion

Number of Roots Parameter

It is well known that NAA induce the plants to produce roots and several studies proved that use of NAA improves plant root formation. Particularly NAA largely used for roots formation.

The results of our study figure (6) showed that T4 treatment which contains 1mg/l NAA without addition of BA gave the highest number of roots. Furthermore, the

number of roots produced through T4 was significantly higher than the other three treatments which are T1, T2, T3. Whereas the average of number of roots in this treatment arrived to 23 roots. Although T4 treatment produced the highest number of roots but plant growth in this treatment was lower than T2 and T3. However, the number of brunches and leaves was lower than T2and T3 because of the absence of BA. This obtained results proved that use of NAA + BA gave plant growth batter than NAA alone. Addition of NAA increases the number of roots but did not improve the plant growth in general. Our results showed also that treatments T1 ,T2and T4 gave the lowest number of roots and no significant differences were found among those treatments.

Length of Roots

For roots length parameter the results of our study figure (7) showed that all the used treatments were in the same level and no significant differences were among all treatments. These results explained that all the treatments were in the same performance in case of roots length.

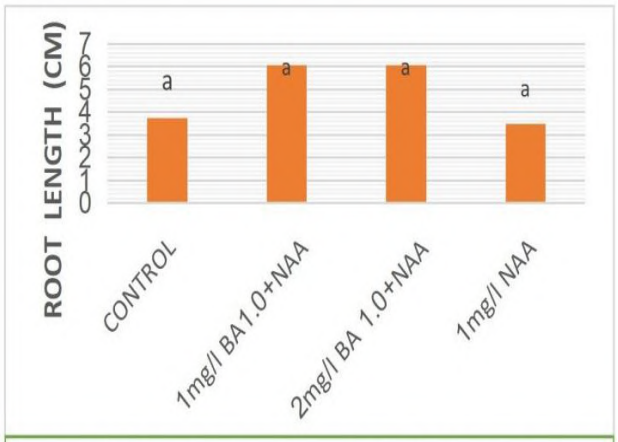


Figure (7) Effect of plant growth regulators on roots leng

5 Conclusion

Ginger plant showed a medical importance. The plant used in human disease recovery. Thereby more quantities are needed. Propagation of ginger plant faces problems especially with plan diseases that is why in vitro Micropropagation extremely needed. Our study proved that use of plant tissue culture technology is clearly successful. The results of this study showed that addition of BA growth regulator improves the plant growth in general and the treatments supplemented with BA gave the best results.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Detection of *MUTYH* gene mutations in hereditary colorectal cancer Libyan families

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A B S T R A C T

Colorectal Cancer (CRC) has become one of the most dangerous yet spreadable cancers, as a result of many wrong behaviors people do, in addition to being a hereditary disease. However, Libya was not included in a lot of studies due to the lack of adequate studies on infected CRC Libyan patients, thus this study aims to detect mutations in the *MUTYH* gene in Libyan families who heredity colorectal cancer. The study included 20 blood samples collected from (10 patients with hereditary colorectal cancer and 10 healthy people) all of them had a family history of this disease. Genomic DNA was extracted, amplified by PCR, and analyzed for *MUTYH* mutational status by direct sequencing. *MUTYH* mutations were present in 50% (10\20) of all analyzed samples. A total of 10 patients had *MUTYH* mutations in different positions; of which 5\10 (50%) had a deletion A in c.1140, 1\10 (10%) a patient had a substitution mutation in c.1149 C>N, 1\10 (10%) a patient had two type mutations, substitution mutation in c.1154 C>T and insertion CT inc.1153, 2/10 (20%) they had substitution mutation in c.1237 G>R, 1\10 (10%) a patient had substitution mutation in c.1140 A>C. This study concludes that indicates that analysis of the MYH gene should be performed in patients with multiple colorectal adenomas, On the other hand, it helped to clarify the type and frequency of MYH mutations among colorectal polyposis patients in Misurata. This study believes that an enlargement of the MUTYH mutation spectrum resulting from these types of studies will contribute to early detection and the prevention of secondary cancer development.

1 Introduction

Colorectal cancer (CRC) is the second most common cancer in women and the third in men. It ranked second in mortality and third in incidence among cancers worldwide in 2020 (Olovo, *et al.*, 2021). Most cases (about 95%) of the CRC are sporadical, Familiar CRCs are less common (about 5%) and occur when gene mutations are passed within a family from one generation to the other. In these cases, mutated genes (germline mutation) are inherited (Centelles, 2010). *MAP* is characterized by the presence of adenomatous polyposis of the colorectal and an increased risk of CRC (Jasperson *et al.*, 2010). The majority of changes found in the *MUTYH* gene are missense mutations, of which Y179C and G396D (previously annotated as Y165C and G382D) represent approximately 73% of *MUTYH* mutations. *MYH*-associated polyposis (MAP) is an autosomal recessive condition, associated with the development of multiple adenomas and carcinomas of the colon and rectum (Hitchins *et al.*, 2005). It is caused by biallelic mutations in the *MUTYH* gene (also referred to as *MYH*) (Jasperson *et al.*, 2010). *MUTYH* gene consists of 16 exons and is located on chromosome 1p34.3-p32.1. *and it* encodes a DNA glycosylase involved in base excision repair (BER) of 8-oxoG:A mismatches caused by oxidative

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DNA damage (Jansen, *et al.,* 2014). The Occurrence of such mutations in oncogenes and tumor suppressor genes drives colorectal carcinogenesis and is associated with the development of colonic polyps (Pitroski,, *et al*., 2011). Functionally, *MUTYH* helps prevent G:C to T:A transversion caused by oxidative stress to highly mutagenic DNA bases (Jasperson *et al.,* 2010). Study between 2014-2015, which aimed to detect the presence of genetic mutations of the *MUTYH* gene with an increased risk of developing CRC It was also observed that the frequency of *MUTYH* p.Y179C mutation is higher among CRC patients than *MUTYH* p.G396 mutation (Jansen, *et al.,* 2014). In 2011 a study was conducted in North Africa that aimed to determine the occurrence of a mutation c.1228dup - 1227 in a group of *MUTYH* patients and assessment of a founder effect within a group of 36 families had MAP, 11 families were found to have a homozygous mutation c.1228dup-1227.These families came from different countries (Algeria, Tunisia, Morocco, and Portugal). The frequency of c.1228dup_1227 is the highest (6.78% vs. 5.4%) of the mutations, and several studies also have shown two closed mutants (Y179C and G396D) are similar to about 80% of MUTYH allelic variants of Europeans Ethnic and geographic differences have been observed in the spectrum of mutations in this gene (Levre, *et al.,* 2011). In the study (2019), samples were collected from 150 Jordanian patients with colorectal adenoma and 150 individuals without cancer with no previous history of polyps. Sanger DNA sequencing of the *MUTYH* gene was performed. The data showed a high prevalence of two mutations in the germline (g.87C>T and c.1264G>C) while *MUTHY* among the affected Jordanians *(Mahasneh, et al., 2019).*

The c.1227-1228dup GG mutation, in a homozygous state, was also found in 13 patients with CRC in the study which evaluated a germline variant of the MUTYH gene, and is an indication to be considered in the testing of the MUTYH gene in patients with hereditary familial polyposis (Kdissa, *et al.,* 2020).

Libya was not included in a lot of studies due to the lack of adequate studies on infected CRC Libyan patients, thus this study aims to: Detection of mutations in the *MUTYH* gene in Libyan families have heredity colorectal cancer.

2 Materials and Methods

Sample Collection

The blood samples were collected from (10 patients with hereditary colorectal cancer and 10 healthy people) all of them had a family history of colorectal cancer. The samples were collected between May and June 2022 in Misurata Central Laboratory. Those samples were stored at -20 ˚C until DNA was extracted.

DNA Extraction

Genomic DNA was extracted from blood samples, using (MagicPure^{TM}Blood Genomic DNA Kit, TransGen, Chinese) according to the manufacturer's instructions.

The Assessment of DNA Quality and Quantity

The DNA concentration was spectrophotometrically assessed using thermo scientific NanoDrop machine (Thermo Fisher Scientific, America). The quality of extracted genomic DNA was examined by loading on agarose gel, to check the integrity of DNA.

Gel Electrophoresis

The electrophoresis was run at 50V for one and half hours. The gel was exposed to UV light using Bio spectrumTM 500 imaging system (UVP) and then photographed using a Multidock-it digital imaging system.

Polymerase Chain Reaction (PCR) Conditions

After extracting the genomic DNA from the samples, the specific region in exon 14 of the target gene (*MUTYH*) was amplified by PCR using the primers (Forward.:3-GGCAGTGGCATGAGTAACAA-5 and Reverse 3-AGAGCAGCTTCAGCGCAAG-5). The PCR reaction was run with the following program: 95˚C initial denaturation for activating the Taq DNA polymerase for 3 minutes in 1x cycle, denaturation at 95˚C for 30 seconds in a 35x cycles, annealing of the primers to the template at 60˚C for 30 seconds in 35x cycles, then extension at 72˚C for 1 minute in a 35x cycles and Step 5final extension at 72˚C for 7 minutes in 1x cycle. The PCR reaction was cooled down for 4˚C. All PCR reactions were done in the Scientific Research Unit at the Misurata Central Laboratory.

Sequencing Analysis

PCR products were sent to the Artha Genomincs Advanced Technologies in Tunisia, for sequencing by Sanger sequencing 7500. The sequencing results were analyzed by using Finch TV and CLC software and Genome browser website to identify mutations in the *MUTYH* gene. A codon number of the mutant gene and it's changed the amino acid sequence were determined by referring to the NCBI/BLAST website.

3 Results

To examine the integrity of the extracted genomic DNA, 5 g of DNA was added to an agarose gel (figure1). Genomic DNA concentration of the 10 specimens included in the study group was between (16.4 ng/µl – 121.8 ng/µl) and 260 nm/280 nm ratios were between (1.90 -1.75)**.**

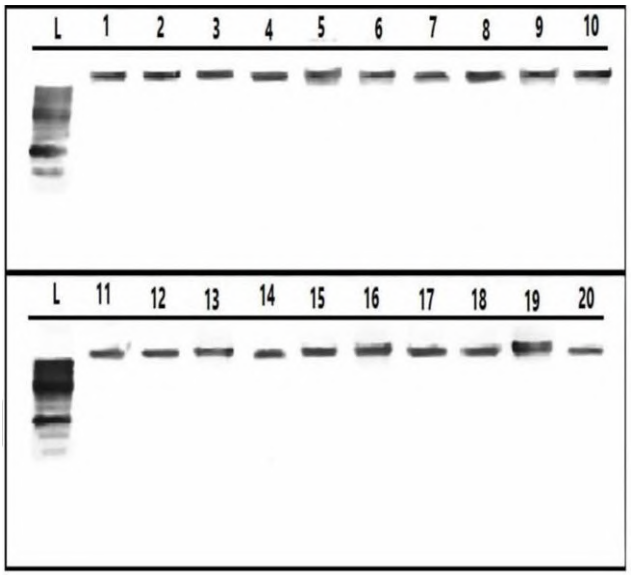

Figure (1): Gel electrophoresis for genomic DNA in 1% agarose gel. L: Ladder 1Kp

The *MUTYH* gene was amplified by PCR using sets of primers described earlier in Materials and Methods. Agarose gel electrophoresis (1%) was run to visualize PCR products and to verify that the products are of the expected size. The PCR products revealed a band of the expected size of *the MUTYH* gene at 149 bp (figure 2).

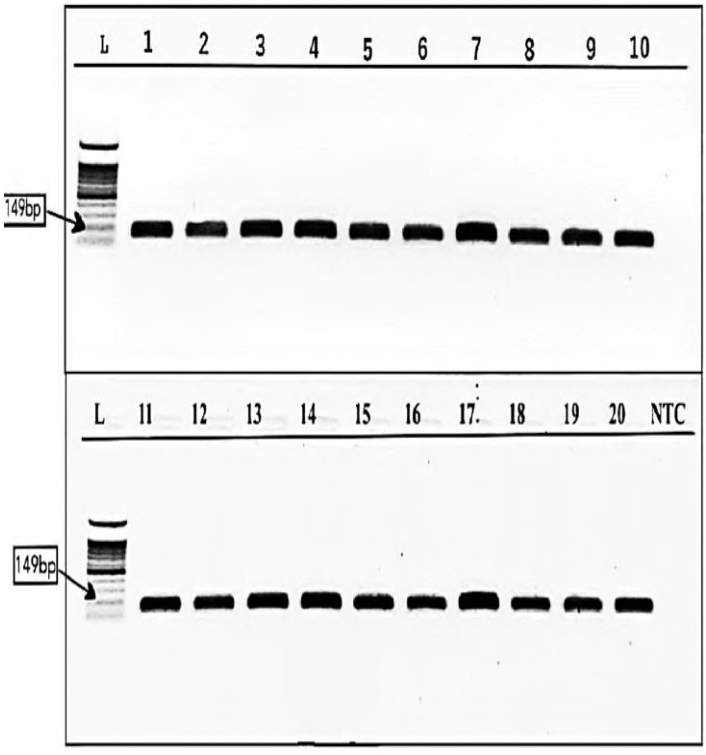

Figure (2): Gel electrophoresis of PCR samples product, the arrow indicates the product with 149bp, NTC: No templet control, L: Ladder 100bp

The type and frequency of *MUTYH* gene mutations are detailed in table (1), *MUTYH* mutations were present in 50 % (10\20) of all analyzed samples. A total of 10 patients had a *MUTYH* mutations in different positions; of which 5\10 (50%), had deletion A in c.1140, 1\10 (10%) a patient had substitution mutation in c.1149 C>N, 1\10 (10%) a patient had two type mutations, substitution mutation in c.1154 C>T, and insertion C inc.1153, c.1140 A > C and c.1237 G > R in proportion (1\10) 10% and (2\10) 20% (Figure 3).

Table (1): Distribution of MUTYH mutation types

N. of samples	Normal sequence	Mutation sequence	Mutation position
1	GCA	GAA	c.1140 A>C
5	GAA	G-A	c.1140 del A
1	CCT	NCT	c.1149 C>N
1	TCC CTC	T**C**CC TTC	c.1153 Ins C c.1154 C>T
2	ARA	AGA	c.1237 G>R

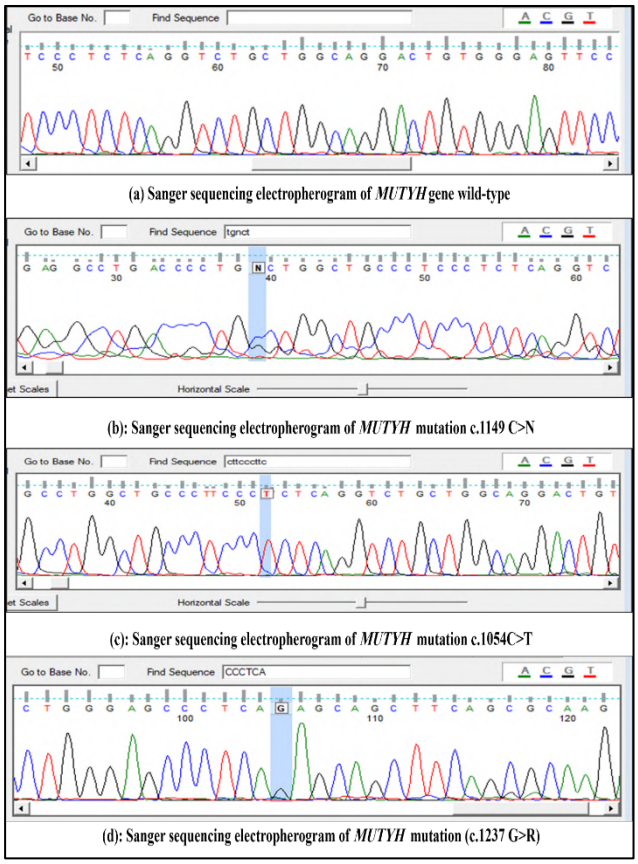

Figure (3): Sanger sequencing electropherogram of *MUTYH* gene.

4 Discussion

This study analyzed *MUTYH* mutations in heredity CRC of Libyan patients in whom CRC incidence and mortality are one of the highest in the Middle East and North Africa, and it is still increasing (Lefevre, et al., 2011). In this study, samples extracted from blood of 20 Libyan patients with CRC were screened for mutation in *MUTYH* gene by using PCR direct sequencing. *MUTHY* mutations were identified in 50% of all heredity CRCs in this study group of patients with CRC. This finding was in a good agreement with previous reports which identified *MUTHY* mutations in 73 % of heredity CRC patients (Sampson, *et al.,* 2005). The frequency of *MUTYH* mutations in the study group did not differ when compared with those of most other studies in the British, Italian, American, Portuguese, and Dutch populations (Sampson, *et al.,* 2005, Cheadle and Sampson, 2007). Previous Arabian studies that enrolled a relatively large number of patients and included patients with different stages of the disease, reported *MUTYH* mutation rates of 80%, 30% in Tunisia (Mahasneh, *et al.,* 2019, Abdelmaksoud-Dammak *et al.,* 2012), 40 % in Portugal (Isidro, *et al.,* 2004), and 95% in Egypt (Elsaid, *et al.,* 2017).

In the study conducted by Afaf Elsaid *et.al.* in 2016 to detect the presence of genetic mutations of *MUTYH* gene with an increased risk of developing CRC, samples extracted from blood of 120 Egyptian patients with CRC, shown that 15 samples had the *MUTYH* mutation and 105 samples negative for *MUTYH* mutation (Elsaid, *et al.,* 2017), where 15 patients also had a family history of CRC thus, a strong association between *MUTYH* mutation and an increased risk of CRC cancer among Egyptian patients, which agree with this study. It was also observed that the frequency of MUTYH p.Y179C (c.536A) mutation is higher among CRC patients than *MUTYH* p.G396D (c.1187G) mutation (Elsaid, *et al.,* 2017), in this study was detected mutation (c.1149C) in same exon.

In 2004 a study was conducted in Portugal in which the biallelic germline strain in the *MUTYH* gene was studied in 53 Portuguese patients with multiple or classic colorectal adenomas and 21 patients had a biallelic germline mutation. New Portuguese-specific mutations in different patients (c.1186 -1187insGG), (c.503G& A) and (c. 340T&C) (Isidro, *et al.,* 2004). These results agree with this study but different position of mutations.

There could be a relatively large number of factors that could be linked to the variations in the observed frequencies of *MUTYH* mutations in the different studies. Among these are using different methodologies for detection of the mutations with different ranges of sensitivity and specificity. Environmental factors have a major impact on different populations with diverse lifestyles, dietary habits, and variable exposures to carcinogens. In addition, using different numbers and kinds of samples, which might serve as another reason for such diversity. Couples with this in the variation in the carcinogen metabolizing genes in different population groups.

5 Conclusion

This study contributes to support the existence of Heredity Colorectal Cancer and indicates that analysis of the MYH gene should be performed in patients with multiple colorectal adenomas, particularly in those with family history of horizontal transmission of the disease. On the other hand, it helped to clarify the type and frequency of MYH mutations among colorectal polyposis patients in Misurata. Although this study showed these mutations in a small region in the MUTYH gene, whole gene sequencing screening procedure in MUTYH is highly recommended in familial colorectal cancer patients. Additional studies will be helpful in identifying new mutations.

Disclaimer: The article has not been previously presented or published and is not part of a thesis project.

Conflict of interest: There are no financial, personal, or professional conflicts of interest to declare.

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An environmental study of some water characteristics of Al-Anaba desert lake in southern Libya

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A B S T R A C T

This study aimed to estimate the physical and chemical properties of Al-Anaba desert lake. The study found that the average temperature ranged between 8.15 and 15.3. The pH rates of the water surface of Annaba were close to the general average of 9.54. The dissolved oxygen ranged from 3.97 to 6.54 mg/l in both the west and south of the lake, respectively. There were no differences in the concentrations of total dissolved salts (TDS) with a general average of 49072 mg/l. The study showed differences in the sodium element concentration from 6.51 mg/l to 8.61 mg/l. There were clear differences in the concentration of the potassium element in the different directions of the lake water with a general average of 0.716 mg/l, which is higher than the concentration of the element in clean sea water (0.38) mg /l. The study showed clear differences in the concentrations of phosphate ions with a general average of 5.28 mg /l. Additionally, the lake is home to various animals such as birds, sea geese, insects like mosquitoes, and aquatic organisms such as brine shrimp. Human activities have affected the succession process in the lake, leading to the growth of the bottom and a reduction in the area of the lake.

1 Introduction

According to various sources, including the World Commission on Environment and Development, the Brandt Land Commission, and the Rio conference on development and the environment, sustainable development is seen as crucial to addressing environmental degradation and other developmental challenges facing the world. It is believed that meeting the needs and desires of humanity while protecting the environment is essential for successful and sustainable development. This is supported by research from Al-Saadi (2009) and the APHA, AWWA, and WPCF (1975) which indicates that a change in behavior towards the environment is necessary to ensure the continuity of the development process. Tourism planning is a crucial stage in the development process that can increase the benefits of tourism while avoiding its negative effects. Therefore, tourism planning is the best way to achieve sustainability in tourist destinations. Desert lakes can be seen in the south of Libya in hollow spaces between large sand dunes. Some of these lakes are usually dry and surrounded by dense vegetation that provides them with natural protection from sand encroachment. One of these lakes, known as "Grape," changes colors throughout the year, resembling the color changes of a grapefruit from birth to maturity. Unfortunately, many of the desert lakes in southern Libya are neglected, which decreases the quality of the desert tourism experience. However, Lake Grape has become a popular tourist destination visited by many groups. Tourism can have a direct impact on the quality of the lake's water and biodiversity. It is important to note that many people do not realize the importance of biodiversity in aquatic ecosystems, particularly in desert lakes. This paper aims to estimate some physical and chemical properties of Lake al-Anaba's water, compare them to neighboring desert water bodies, and analyze some qualitative and quantitative characteristics of the plants surrounding the lake's surface. The data gathered will form an initial database for Lake al-Anaba (Schelske, 1988).

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2 Previous Studies

Previous studies have focused on the ecosystems of lake waters, as stated by Abd al-Majid and al-Dardiri in 2001. One of these studies was conducted by Richadson and Gene in 1971, which revealed that environmental factors like temperature and humidity contribute to the distribution of vegetation cover and play a crucial role in accelerating the succession processes. Geyh and Thiedig in 2008, and Hatoua *et al*. in 1996, suggested that changes in non-living or living factors in a region can bring about a change in living communities. My father knows about succession, which is an organized development in environmental systems that leads to the establishment of a vital community in place of a previous one. A study by Schelske in 1971 indicated severe unwanted changes in the ecosystem of Lake Michigan. During the summer season, the proliferation of dominant phytoplankton, which require silica, is limited. Kim *et al*. in 2021 reported that hydrosea occurs in aquatic environments such as ponds, lakes, and swamps, where the succession goes through a series of stages. The study by Schelske (1988) examined the historical trends of silica concentrations in Lake Michigan, which indicates a decrease in silica impact due to increased deposition by diatoms, contributing to an increase of organic phosphorus. The study by Burdon and Gonfiantini (1991) focused on geology, hydrology, chemistry, and excess isotopes in the lakes of the Ubari Sand Sea in Fezzan. This study described the origin and formation of one of these lakes (Lake Mandara). In 2004, Manwar discussed in his study about Lake Byoua the factors that stimulate the improvement of food, which increase this process and accelerate the environmental succession process. In another study (Al-Mujahid, 1995), the stages of development of aquatic plants were divided from submerged plants to floating plants, reed swamps and meadows, then trees and climax communities. These are successive stages in the process of evolution, and each stage is characterized by certain types of dominant and distinctive plants.

A study (Jaing.S; *et al* 2004) indicated that the pH of Lake Momo recorded an average capacity of 10, i.e., The water of the lake is alkaline, and its average salinity ranges between 70 and 85 g/kg. As shown by (Mitsch, *et al* 2005). in a study of the qualitative change of water and the effect of this change in wet water systems, he indicated that succession works to change the water quality, and this, in turn, affects the growth of some aquatic plants. A study (Al-Sheikh., 2010). of Al-Asfar Lake in Al-Hasa in the Kingdom of Saudi Arabia indicated the properties of physalis. The chemical characteristics of the lake water indicate that the high concentration of permanent oxygen is associated with an increase in the effectiveness of phytoplankton in the photosynthesis process.

And a study showed (Vijayakumar, 2010) the chemical properties of the surface water of Lake Perumal in India, which showed the values of connectivity that ranged from 362 to 61800 s// cm µ and the PH between 7.2 and 8.1, while the TDS ranged between 254 and 17600 mg/liter, and the nutrients were measured and ranged between 0.829 and 1.942 mg/liter. As for phosphate, it recorded a concentration of 0.019 mg/liter.

(Al-Tani, *et al* 2013) evaluated the environmental succession of Lake Qabroun, and the results showed that the lake is distinguished by its transparency on the eastern side, while the western side is the least transparent, and its pH ranges between 9.25 and 10.31, meaning that the lake water is alkaline, and the conductivity of the lake reached (84320–237860 s/cm) with a high concentration of salts. A study (Al-Mutanan, 2016) showed that the ecosystem of Lake Umm al-Maa in the south of Libya is characterized by extreme chemical, natural, and biological characteristics for environmental and human reasons within its water surface and its vegetation surroundings, which lead to accelerating the processes of environmental succession and the disappearance of the lake. A study (Faraj 2018). on the environmental assessment of water quality and productivity in the northern lakes in Egypt indicated that they represent important points for biodiversity, as they are a haven for migratory birds in addition to thousands of animals, botanical inspirations, and microorganisms.

3 Materials and Methods

3.1 Study Area

Lake Al-Anaba is located Southern Libya in the Wadi Al-Shati area, and it is about 80 km away from it within the Ramlet Zalaf basin, between lines of length (26.944695) and width (12.977196) between the sand dunes, as shown in Figure No. 1. It was located within what was previously known as Lake The Great Fezzan, a giant lake with an area of about 150,000 km2, is a large lake that covers large areas of the Libyan desert, bordered by the Hamada al-Hamra plateau from the north, the Black Mountain and the black Harouge volcanic shield from the east and south, and the Acacus Mountains from the west (Al-Salman, *et al* 2007). Evidence has indicated that the Fezzan region contained sediment deposited from rivers and lakes during the period of the most humid environments. (Drake, *et al* 2008).

Figure (1): A picture of Lake Al-Anaba

3.2 Sample Collection

The samples were collected from the study site (Lake Al-Anaba), where the sites were determined by taking samples from the lake according to the geographical directions (east-west-south-north). Where water samples were collected from these sites for the purpose of identifying the physical and chemical properties using (7) liter plastic bottles with (4) samples with three replicates represented by one sample for each geographical direction (east-west-south-north), botanical samples were taken from Each site was collected and classified according to its types and species, according to the classification found in the Libyan Flora book series. The samples were collected during a field visit to the lake on February 19, 2022, from 10:30 a.m. to 7:00 p.m.

3.3 Estimating the Physical and Chemical Properties of the Lake Water

A. **Temperature (T°C):** The temperature is measured directly during sampling using B. pH and thermometer together (a HQ40D multimeter with two channels).

B. **Electrical conductivity (EC):** The conductivity is measured directly after collecting samples using a laboratory conductivity meter. Model 4310.

1. **pH: (pH** is measured immediately after collecting samples using a pH-Meter) type PHILIPS and model (9421)

2. **Chloride (CL):** The calibration method mentioned in (Al-Sheikh. 2010 No. (408) is used. With a 0.0141N silver nitrate solution using a potassium chromate reagent

3. **Sodium and potassium: (Na, K)** A flask photometer is used to measure sodium and potassium in water according to the method mentioned in (Grasshoff 1976) No. 317,230.

4. **Phosphate: (Po_4)** Phosphate was measured in water samples using a colorimetric method. This method relies on the intensity of the color that forms after adding vanadate-molybdate, and the absorption is measured at a wavelength of 470nm using a UV-Vis Spectrophotometer.
5. **Total Dissolved Salts (TDS):** The total soluble salts of the samples were estimated through conductivity measurements using a previously mentioned device. It was calculated using the equation EC * 0.64 = TDS.

3.4 Estimate the Qualitative and Quantitative Characteristics of the Plants Around the Lake.

A. Measuring plant diversity: Diversity was measured using one of the indicators of relative diversity known as the Simpson index, which is one of the types of control indicators because it weighs the abundance of common species and gives the probability of any two individuals taken randomly from the community to which several species belong.

The Simpson Index states the following law: SDI = 1-D

$N(N-1)\ \sum D= n(n-1)$

Where n = the number of individuals in one species

N = the total number of individuals for each species

D = Diversity in general

means sum $=\sum$

SDI = Simpsons diversity index.

B. Estimation of plant density: The plant density was determined in one of the plant samples using the environmental square method (100 * 100 cm) in order to determine their types and prepare them to calculate their density per unit area (plant/mm squared) from the relationship: Vegetation cover density = a number of community members per unit area of the study area (1 m2). (Goldsmith, et.al. 2016) & (Zhuang, 2020)

C. Estimation of plant abundance: The abundance of plant species was estimated by taking plant samples using the environmental spatial square (100 * 100 cm) and counting the plants inside. The abundance of each plant species was calculated using the following mathematical relationship:

The percentage of abundance for each species is equal to the number of individuals of one species x the total number of species x 100, and by comparing these percentages with the environmentally approved percentages in calculating the abundance of species for the community shown in the following table mentioned in (Florence, *et al* 2012) & (Al-Salman, *et al* 2007).

Degree of abundance Percentage scientific term
Dominant type < 95% of the total
Type Abundant > 51-95%
Common type 10 - 50%
Frequent type 1 - 10%
The rare type is 0.5 – 1%
Very rare type 0.5 > %

D. Frequency: Frequency is the number of times the plant appears in a number of sample frames of a similar area.

of it as a percentage. It depends on both density and distribution. Thus, changes in plant abundance and distribution can be measured.

Frequency calculation: the number of samples in which the type appeared Frequency x the total number of samples used x 100

3.5 Identify the Animal Species Present in the Study Area

3.5.1 Aquatic Animal Species: Water samples were taken from each site according to the division of the four sides, east, west, south, and north, in 7-liter bottles.

3.5.2 Non-aquatic Animal Species: Species were identified by eye observation, i.e., what was seen in the study place, and some species were photographed with a mobile phone camera.

3.5.3 The impact of Human Activities: the observance of funerals was recorded at the studied sites, and any effect of human activities on the lake environment was observed using the mobile phone camera.

4 Results

4.1 Physical and Chemical Properties of the Lake Water

4.1.1 Temperature: The results of the study showed It is shown in figure (2) that the average temperature fluctuated between 15.3 and 15.8 in both the south and west of the lake, respectively.

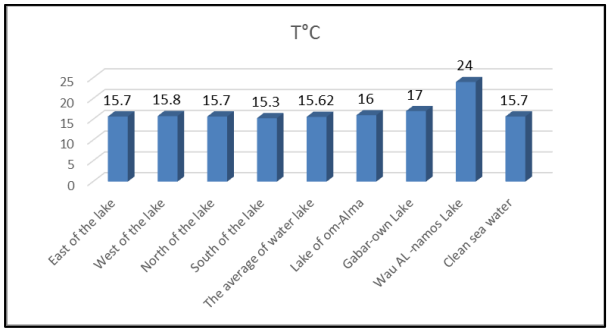

Figure (2): shows the temperature concentration in the study area.

4.1.2 pH: The results of the study showed, as shown in Figure (3), that the pH rates within the water surface of Anaba were close to a general average of (9.54).

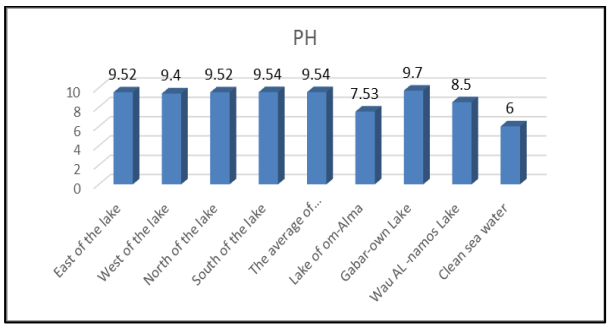

Figure (3): shows the pH concentration in the study area

4.1.3 Electrical Conductivity:the results of the study were recorded as shown in Figure (4). The general average of the conductivity values in Lake al-'Anaba reached 7682 ds/m^2, which

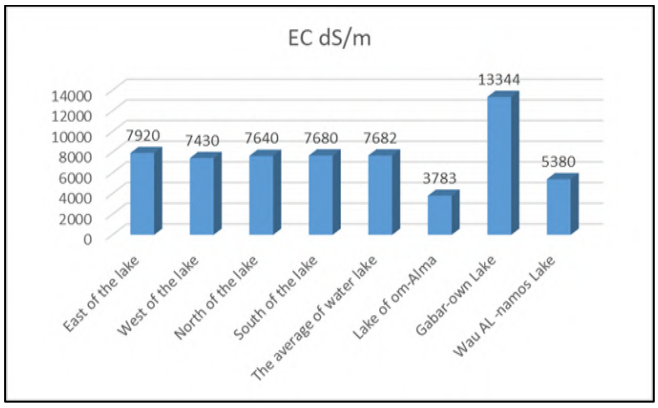

Figure (4): shows the concentration of electrical conductivity in the study area

4.1.4 Dissolved Oxygen: The results of the study, as shown in Figure (5), recorded a fluctuation in the dissolved oxygen values in the water of the lake from (3.97) to (6.54) mg/liter in both the west and south of the lake, respectively, with an average general (5.3) mg/liter,

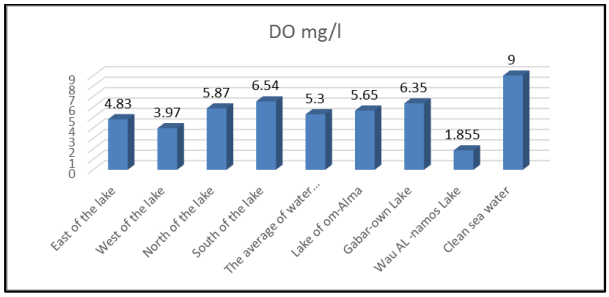

Figure (5): shows the concentration of dissolved oxygen in the study area

4.1.5 Total Dissolved Salts: The results of the study were recorded as shown in Figure 6. The water surface of Anaba has a general average of 4907.2 mg/l.

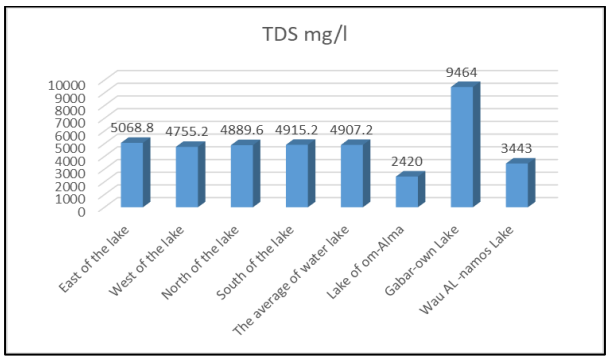

Figure (6): shows the concentration of total dissolved salts in the study area

4.1.6 Chloride: The results of the study are shown in Figure No. 7. Convergent values of chloride concentrations in various directions inside the lake with a general average of (29.5) mg/liter

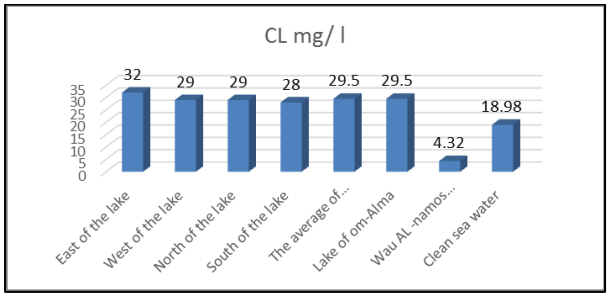

Figure (7): shows the concentration of chloride in the study area

4.1.7 Sodium: The results of the study are shown in Figure No. 8. Differences in the concentration of the sodium element from (6.51) mg/liter to (8.61) mg/liter in both the south and west of the lake, respectively, have a general average of (7.77) mg/liter,

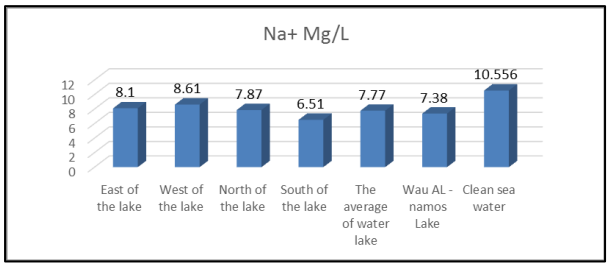

Figure (8): shows the sodium concentration in the study area

4.1.8 Potassium: Potassium in the different directions of the lake water in both the south and west of the lake is (0.545) and (0.841) mg/liter, respectively, with a general average of (0.716) mg/liter,

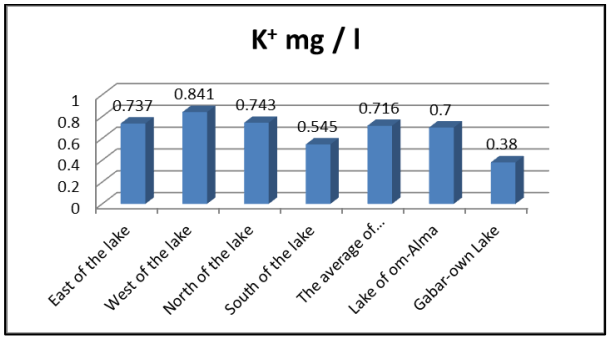

Figure (9): shows the concentration of potassium in the study area

4.1.9. Phosphates: The results of the study, as shown in Figure No. 10, showed clear variations and differences in the concentrations of phosphate ions in the water of Lake al-Anaba, where the water in the west of the lake recorded the lowest reading compared to the east of the lake (4.29) mg/liter and (7.35) mg/liter, respectively, with a general average of (5.28) mg/liter.

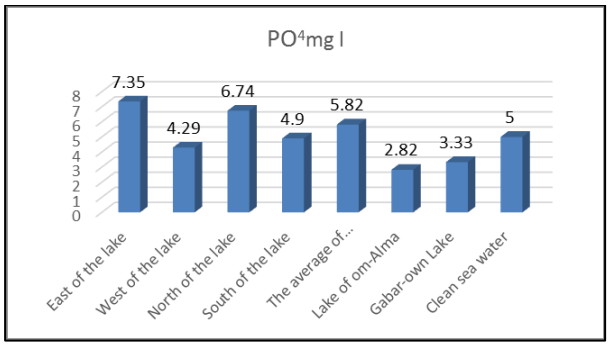

Figure (10): shows the concentration of phosphate in the study area

4.2 Specific Characteristics of Plants Around the Lake: Plant samples were taken from each site, collected, and classified according to their species, according to the classification found in the series of Libyan Flora books. The dominant plant on the study site is the reed plant. Along with this plant, there are other plants, some of which are densely distributed, some of which are mediumly distributed, and some of which are found in small numbers. The plants that are found on the study site are:

A- Cymbopogon Citratus plant: a perennial plant, the stem is an unbranched, smooth rhizome, with equal leaves, tapering at the tip, the inflorescence is compound, it has two unequal stems and the lower brachial is longer than the two stems. It blooms during summer and spring, and it is one of the semi-submersible plants belonging to the Poaceae family.

This is distinguished The plant grows in shallow and moist areas.

B-Pinus halepensis is a large perennial tree, reaching a height of (11-16 meters), the stem is smooth, with a pale green or gray color, and the leaves are very small and serrated, with a sheath of (1.5-2 mm) long. Bleached pink, blooms from (8-11) months. This plant belongs to the Tamari cacao family. It is considered one of the plants that tolerate salinity and stabilize sand dunes.
C- A triplex halimus : plant: has a creeping or oblique rhizome stem, its length is (40-150 cm), devoid of hairs, i.e. the stem is smooth and covered at the base with a sheath leaf, the leaves are flat tapering with a ribbon blade and a dense peripheral inflorescence, the spike is semi-clustered, compound, the length of the spikes is (5-6).

D- Phoenix dactylifera: perennial trees with a stem that reaches a height of 40 meters. The old leaves are protective and located at the bottom of the tree. As for the leaves and flowers, they are found at the top, while the leaves at the top are smooth green in color, reaching a height of (30-50 cm), petiolate, feathery, and containing thorns. The fruits are a drupe that blooms during the months of 4 and 5. This plant belongs to the Plamceae family.

E Ziziphus Christi plant: a relatively small shrub with a length of 40 cm. It has very small leaves and stores water in large quantities. It has yellow flowers and very small seeds. Its roots are thin and not very deep.

F- Rhus ascendance plant: a juicy plant that stores water mainly in the leaves and in the stems that grow in the ground. (Ivanova, 2018) & (Ivanova, 2015).

4.3 Quantitative Characteristics of the Vegetation Around the Lake:

Vegetation density and diversity were calculated and determined and vegetation abundance and frequency were estimated for all species We find that the A triplex halimus plant is prevalent on the northern side of the lake and is the highest frequency, density, and abundance, than Phoenix dactylifera and Pinus halepensis, while we find that the Cymbopogon Citratus plant is prevalent in the southern side Followed by each of the A triplex halimus and Phoenix dactylifera and we find that the A triplex halimus plant is the only plant was dominating in the western side, and the study showed that the plant A triplex halimus, Cymbopogon Citratus plant and Pinus halepensis are the most frequent and dense in the eastern side as shown in figures (11) (12) (13) (14)

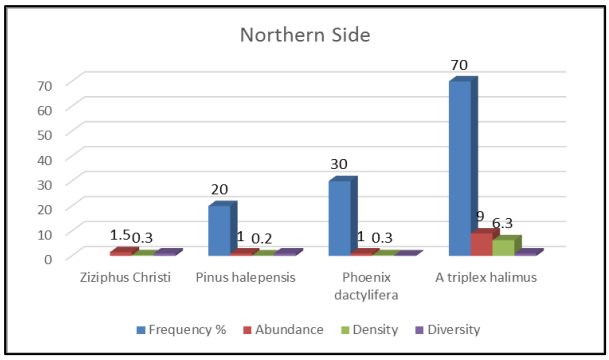

Figure (11): shows the Qualitative and quantitative characteristics of the plants of the northern side

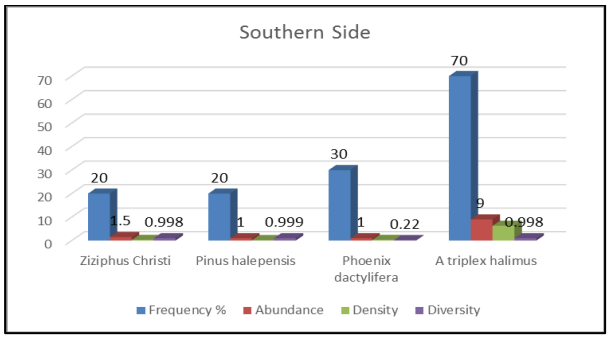

Figure (12): shows the Qualitative and quantitative characteristics of the plants of the southern side

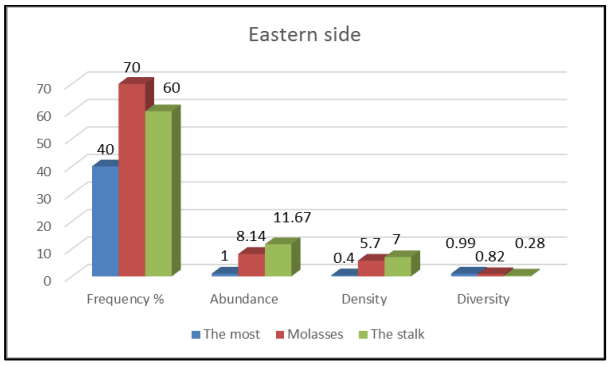

Figure (13): shows the Qualitative and quantitative characteristics of the plants of the eastern side

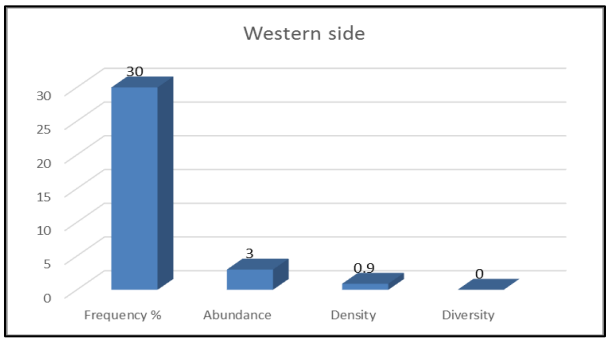

Figure (14): shows the Qualitative and quantitative characteristics of the plants of the western side.

E. Species and aquatic and non-aquatic organisms around the lake:

The western and eastern sides of the water surface of Lake Al-Anaba recorded the presence of brine shrimp, a crustacean animal that lives in the salt water of the type Artema salina, which is a primitive gill-legged crustacean (Branchiopoda) without a shell, while the western side recorded the highest number density of brine shrimp, which amounted to (50 individuals / 5 Liter) and from the eastern side, (20 individuals / 5 liters) were recorded. As for the southern and northern sides, no individuals were recorded. The results showed the presence of some birds, sparrows, and sea geese, but in very few numbers, and the presence of mosquitoes in a large number.

4.4 Human Activities Around the Lake:

It was observed that there was waste dumped around the lake from the northern side, and this indicates that visitors stay on this side of the lake. From the lake, there are paths heading from the top to the inside of the lake, as well as the presence of car tires near the edge of the lake on the southern and western sides, which explains the reason for the decrease in vegetation cover in it.

5 Discussion

5.1 Physical and Chemical Properties of the Lake Water

Temperature: The water temperature is one of the specific environmental factors affecting the water environment. The temperature in the water is affected by the different depths and locations relative to the latitude circles. The change in temperature leads to changes in water masses and their circulation, which has a significant impact on aquatic life (Al-Saadi 2009). and compared to the general average temperature of other desert lakes, its rates were lower (15.6), especially Lake Waw al-Namos, which has a natural nature. The hot volcanic temperatures reached 24 degrees Celsius (Hatoua, *et al* 1996). and these results are consistent with the studies of (Al-Mukhtar, *et al* 2002) and (Nino Ambarak *et al* 2021). (17), (16), respectively.

The pH value is affected by many components, such as the bottom soil, the shape of the water basin, the temporary water content of gases and ions, and the presence of aquatic plants. Low and high pH values harm the growth rates and fertility of aquatic organisms. Fluctuations in the pH values are large and clear during the hours of the day. The pH suitable for living ranges from 5.5 to 10, and the most productive water is low-alkaline. (Mihir Pal, *et al* 2015) & (Al-Saadi 2009). meaning that the alkaline water is not much different from Lake Qabroun (9.7), and its rates are higher than Lake Waw an-Namos, Umm al-Maa, and Clean Sea Water (8.5), (7.5), and (6), respectively. These results agree with the studies of (Al-Mukhtar, *et al* 2002) & (Nino Ambarak *et al* 2021). (9.7) and (7.5), respectively

Electrical Conductivity Salinity is expressed by the amount of conductivity, which is the sensitivity and ability of water to electrical conductivity. It is characterized by the dissolved ionic concentrations in it and the quality of these ions. That is, the increase in the concentration of the percentage of these ions means an increase in their electrical conductivity. (Florence, *et al* 2012). The general average of the conductivity values in Lake al-'Anaba reached 76.72 ds/m^2, which is lower than the value recorded in Lake Qabroun (133.84 ds/m^2) and higher than the lakes of Waw an-Namos and Lake Umm al-Maa (53.8 ds/m^2) and (37.83 ds/m2), respectively. These results differ with the studies (Nino Ambarak *et al* 2021) & (Al-Saadi 2009). (133.8 ds/m^2) and (37.83 ds/m2), respectively.

Dissolved oxygen values are affected by several factors, including the abundance of aquatic plants, wind speed, and water currents, all of which lead to an increase in the solubility of oxygen in the water. There is an inverse relationship between dissolved oxygen and temperature(Al-Manhrawi, 1997). The values obtained are much higher than the value recorded in Lake Waw Al-Namous and much lower than the permanent oxygen in clean seawater (1.855) and (9) mg/L, respectively. These results are consistent with studies (Goldsmith, *et al*. 2016) & (Jaing.S; *et al* 2004). (6.35) and (5.65), respectively

Total dissolved Salts: Salinity is one of the environmental factors with large and important fluctuations affecting the distribution and spread of aquatic organisms and determining the size of the biological community. The types and numbers of organisms in the water differ according to the salinity. Salts enter the freshwater environment through groundwater from the erosion of rocks in the air and are transmitted by wind and rain (Drake, *et al* 2008) & (Ivanova, 2015). The concentration of salts in the water of Lake Anba is higher than the water of Lake Umm al-Ma and lower than the concentration of salts in Lake Qabroun (24.2) and (94.64) mg/L, respectively. These results differ from studies (Grasshoff 1976), (Nino Ambarak *e .al* 2021), and (Shen, *et al* 2017) (94.64 mg/L) and (24.2 mg/L), respectively.

Chloride It is an increase in the concentrations of elemental chloride in clean seawater and Lake Waw an-Namos (18.98) and (4.32) mg/l, respectively. These results agree with the study (Al-Mukhtar, *et al* 2002) & (Al-Salawi, 1989). (29.5 mg/th and in surface waters in the form of sodium chloride, potassium, and calcium Al-Matmani M.N (2010). and the results of the study

are shown in Figure No. 7. Convergent values of chloride concentrations in various directions inside the lake with a general average of (29.5) mg/liter It is an increase in the concentrations of elemental chloride in clean seawater and Lake Waw an- Namos (18.98) and (4.32) mg/l, respectively. These results agree with the study (Al-Mukhtar, *et al* 2002) & (Al-Salawi, 1989). (29.5 mg/L), and differ from the study Al-Matmani M.N (2010). (4.32 mg/L).

Sodium The concentration of sodium in the water of Lake Anba is slightly higher than the concentration of sodium in Lake Waw Al-Namous (7.38) mg/L and less than the concentration of the element in clean sea water (10.556) mg/L. These results are consistent with the study of Al-Matmani M.N. (2010) and (Al-Saadi 2009) (7.38) mg/L.

potassium is found in lower concentrations than sodium in water (Ivanova, 2018) & (Ivanova, 2015).The concentration of potassium in the water of Lake Anba does not differ from the concentration in Lake Waw Al-Namous (0.700) mg/L and an increase in the concentration of the element in clean sea water (0.38) mg/L. These results are consistent with studies (Al-Mathani 2010) and (Al-Mukhtar *et al*. 2002) 0.716 mg/L and 0.7 mg/L, respectively.

Phosphate slightly soluble in water. Phosphate derivatives are used in the form of phosphate fertilizers. (Mara'I, 2003).Phosphate concentrations are high compared to clean sea water, Lake Qabroun and Umm El Maa (5) (3.33) and (2.28) mg/L, respectively. These results are consistent with studies (Al-Mukhtar *et al.* 2002) and Embarek *et al*. 2021) (3.33) mg/L and (2.82) mg/L, respectively.

5.2 Specific and Quantitative Characteristics of the Vegetation and the Lake

There is a diversity of vegetation, namely (reeds, palm trees, tamarisk, damran, and marsa). The reed plant was the most widespread and abundant due to its competitive ability and resistance to environmental conditions and the characteristics of the roots that enabled it to crawl towards the lake waters, in addition to the fact that the environmental conditions are considered more suitable for this plant. (Faraj 2018)

6 Discussion

Species and aquatic and non-aquatic organisms around the lake:

The presence of animals that live in and around the lake, namely birds, is an indication of their high resilience and resistance to harsh environmental extremes. (Faraj 2018) In addition to the presence of Artemia, studies have indicated that Artemia is found in salty water bodies and its presence is considered a vital indicator of the presence of bacteria and phytoplankton as its preferred food, and this is consistent with (Ivanova 2018).

6 Conclusions and Recommendations

6.1 Conclusions

Through a study of Lake al-Anaba, the following conclusions can be drawn:

- The ecosystem in Lake al-'Anaba is characterized by special chemical, physical, and biological characteristics and is exposed to many changes for environmental and human reasons in its water surface, vegetation, and terrestrial surroundings.

The characteristics of the water in the lake indicate that it has high conductivity, which indicates high salinity due to the high concentrations of total dissolved salts (TDS), accompanied by the presence of concentrations of nutrients such as phosphates, which contribute to nutritional enrichment and accelerate environmental succession processes, as well as the effect of dissolved oxygen and its role on organisms. Aquatic living.

- The high rates of sodium and potassium in the water are evidence that the water contains these elements, resulting from the geology of the rocks under the lake and their impact on the life of living organisms and their life cycle.

- The pH was high, evidence of the alkalinity of the lake water, which provides a suitable environment for the living organisms and algae that live in it. The lake was called the grape because of its different color during the seasons of the year, as is the case in the color of grapefruits from birth to maturity, which may be due to the quality of algae and the biodiversity within the water. the lake

- The vegetation cover was characterized by a limited diversity, where the existing species were (reeds, dais, palm trees, tamarisk, damran, and anchoring).

- The vegetation extends around the lake (20 m) and the reed plant was the most dominant due to its competitive potential and rhizome characteristics that enabled it to crawl towards the lake water.

- It also found living animal organisms, and this ecosystem was characterized by the presence of animal species, the most important of which are birds. During the study, the presence of artemia was observed, and

this gives an indication of the effect of nutrients and dissolved oxygen, which is considered to have a specific role in its life cycle and its adaptation mechanism.

- There are many negative effects of human activities around the lake, represented in the pollution of the lake's surroundings with waste and the destruction of vegetation cover, all of which contribute to accelerating the occurrence of the succession process, which in turn contributes to the disappearance of the lake with what it represents as an environmental, cultural, and touristic landmark.

6.2 Recommendations

Continue to study the physical and chemical properties of the water in the southern lakes on a regular and continuous basis.

Carrying out an extensive study of the environmental community of the benthic lake because of its important role in preserving and reducing the growth of the benthic lake. Tightening and activating measures to protect the lake from all manifestations of encroachment on it, such as practices of deliberate burning of vegetation cover and other manifestations of vandalism.

Develop a scientific plan to stop the encroachment of plants and weeds into the lake.Making suitable paths for going down to the lake water, including the lack of movement of sand towards the lake water studying the climatic environmental factors and establishing a meteorological station around the lake to track climate changes in the lake because climate changes greatly affect the continuation of the lake and the preservation of its presence.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Spectroscopy of Alpha-Particles Using the Thermally Diffused p-i-n Detector

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A B S T R A C T

The goal of this experimental work is to learn more about the characteristics of alpha particles, how they interact with matter, and their range. A thermally diffused detector and a triple alpha source with the isotopes ^{239}Pu, ^{241}Am, and ^{244}Cm were employed in the experimental work. The primary aim was to obtain three main energy peaks and then determine the absolute activities of these isotopes. The absolute activities were calculated, and they were 1652.38±40.6 Bq, 936.38 ±30 Bq, and 706.8±26 Bq for ^{239}Pu, ^{241}Am and ^{244}Cm respectively. The second aim was to calibrate the Multi-channel analyser at lower energies known as attenuation output using the initial pulser peak that covered the Am^{-241} peaks at an energy of 5.49MeV. The FWHM was estimated using counting statistics to be 11.23.35 kev. Then, it was determined when the alpha emission for three isotopes started to stop, using an extrapolated range of alpha particles. In order to draw the graphs of each radioisotope's peak energy versus air pressure and the FWHM versus six spectra of gas pressures, air pressures of various m-bar air pressures were measured. The procedure should ultimately be completed by measuring the intensity of -particles and documenting the peak count rate for a particular element, in this case ^{244}Cm.

1 Introduction

The radiation emitted by naturally occurring materials has been shown to have the least penetration when it comes to alpha particles. Coulomb repulsion is the cause of alpha emission. It is crucial for interactions with matter, especially with heavy nuclei, which take place because the disruptive Coulomb force accelerates with increasing size (Knoll et al, 2010). The technique used to assess the energy of particles and their activity is known as alpha particle spectrometry. It could identify the radiation's source. In light of this, charged particle spectrometry dominates radiation physics (Lilley, 2013).

Alpha particles consist of one proton and two neutrons. They have a strong electron charge and high energy. They have substantially greater masses when compared to beta and gamma particles. They are not particularly piercing, though. For instance, 5 MeV of -particles can pierce common paper with a penetration depth of only 3.6 cm in air (Knoll et al, 2010). The emission of alpha particles produces elements that have a high atomic number. For example, alpha particles are emitted throughout the decay of radium.

$^{266}Ra \rightarrow {}^{222}Rn + \alpha$

The next figure shows how radium degrades from a high energy state to a lower energy state, which results in a reduction of protons and the emission of alpha particles.

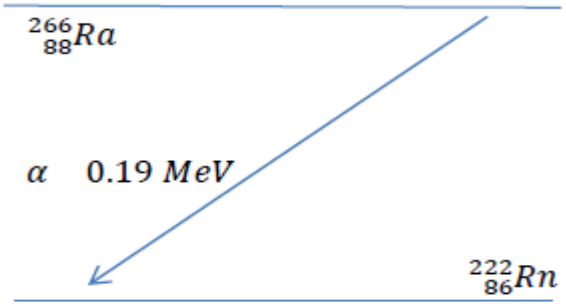

Figure (1): the decay of Ra^{-266}

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2 Theory

In alpha decay, the parent atom ejects a defined daughter nucleus with a double charged particle (4He-nucleus) and a release of heavy particles. Alpha particles can be stopped by a layer of skin or a sheet of paper (Knoll et al, 2010). Also, if an alpha particle emitter is inhaled or ingested, it can cause very serious damage to a human's health. Alpha decay can be represented as (Knoll et al, 2010. Lilley, 2013).

$$^{A}_{Z}X \rightarrow {}^{A-4}_{Z-2}Y + {}^{4}_{2}\alpha + \gamma$$

Alpha particles can interact with either nuclei or orbital electrons in any absorbing medium, such as air, water, tissue, and metal. There is an amount of alpha emission passing through the nucleus that could be deflected with no change in energy (Rutherford scatting). However, defects could occur with a small change in energy or be absorbed by the nucleus, causing transformation. This process is called negligible for alpha particles (Cember, 2009). The most likely process in the absorption of alpha is the ionization and excitation of orbital electrons. Ionization occurs when the alpha particles are close to electron and pull it out from orbit though coulomb attraction, as the result α- particles lose kinetic energy and become slow. They can lose their kinetic energy by exciting orbital electrons with interactions that are inadequate to cause ionization. As the alpha closes to the end of its way, its rate of ionization peaks, and within a very short distance, it stops, collects two electrons, and becomes a helium atom (Cember, 2009).
The absolute activity can be estimated using the equation below:

$$I = \frac{C\alpha}{f} \times \frac{4\pi d^2}{A} \qquad Eq\ 1$$

Where I is the absolute alpha activity, f is the fractional intensity of a particular alpha peak energy, Cα is count rate , A is the area of the detector active surface, which is 1.3 cm^2 and d is the source detector distance, ,which is 1.5cm. The alpha particle range is the distance where it travels in the air and has the lowest penetration because of its short range and attenuation.
The empirical equation below is used to calculate the predicted range of alpha particles:

$$R_\alpha = 0318E^{1.5} \qquad Eq\ 2$$

STP is Standard Temperature and Pressure
MCA is Multi Channel Analyser
Where Rα is the range of alpha particles in air at STP in cm, Eα is the energy of alpha particles in MeV.

Accurate calibration with low energies at the MCA scale is required. The pulsar injection is likely to be used; the width of the pulsar peak is obtained through the MCA. The width of the peak due to statistical noise can only be calculated via the equation below:

$$\mathrm{FWHMtot}_{total} = \sqrt{(\mathrm{FWHM}_{\mathrm{elec}})^2 + (\mathrm{FWHM}_{\mathrm{stat}})^2}\ Eq3$$

Where $\mathrm{FWHMtot}_{total}$ is the width of the ^{241}Am peak which used in the experiment and $\mathrm{FWHM}_{\mathrm{stat}}$ is the width of the pulser peak that is in jested into the MCA (Regan, 2014).
When the pressure increases, the widths of α-peaks increase due to energy straggling, which is an increase in the energy distribution of α-particles reaching the detector due to fluctuations in the number of atomic collisions.
A theoretical prediction may be written in the form:

$$\mathrm{FWHM} = 41.6(\Delta x)0.5\ \mathrm{KeV} \qquad \text{Eq 4}$$

Where is the air-path thickness expressed in mg cm^{-2}. In this experiment, this prediction could be obtained by drawing a graph of the FWHM of the ^{241}Am peak versus the square root of the air thickness (Regan, 2014).

3 Experimental Methods

The thermally diffused p-i-n detector was used to obtain alpha spectrometry, and the triple-alpha source consisting of ^{239}Pu, ^{241}Am, and ^{244}Cm is placed on the source holder at a distance of approximately 1.5cm from the detector within a vacuum chamber. The operating voltage was set at 24 volts, the time live was 300 seconds, the shaping amplifier was 1 sec, the gain was 100, and the vacuum pump was at zero m-bar.
The first spectrum was recorded, and the three main peaks were accumulated.

The MCA was calculated via the three main peaks, and report information was obtained for each peak in order to calculate the absolute activity of three isotopes. To obtain the range of alpha particles, it must calibrate the MCA at lower energies, and that mainly depends on the initial energy pulser that was injected into the MCA and the known attenuation of the pulser output. Then the dates were obtained, graphs plotted, and the alpha range demonstrated. The predicted range of alpha particles to three isotopes was estimated.

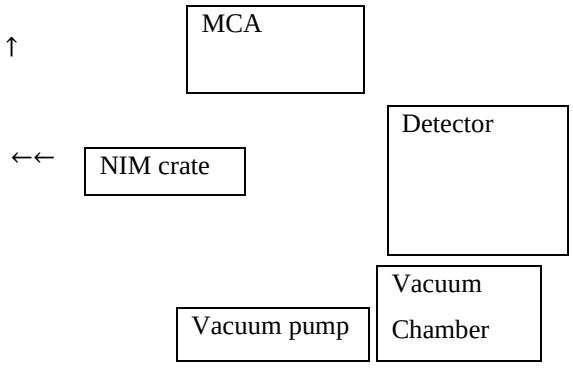

Figure (2): The thermal diffused p-i-n detector and vacuum chamber

4 Result

The three main peaks were obtained on the MCA from the triple alpha source, as will be shown in Appendix 1. Figure 2 shows the calibration between the FWHM of the three main peaks of ^{239}Pu, ^{241}Am, and ^{244}Cm versus their peak energies.

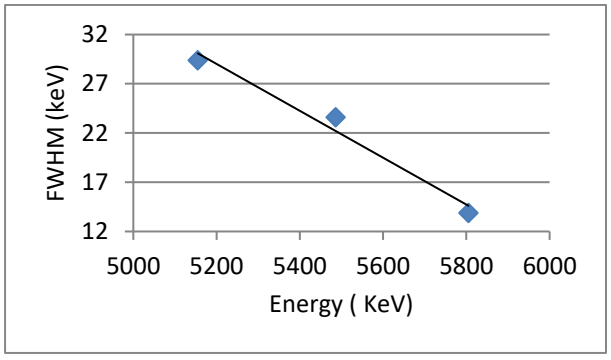

Figure (3): describes inverse relationship between an increase of energy and the decease of the FWHM.

The absolute activities of the three isotopes were determined from equation 1 and are displayed in Table1.

Table (1): the absolute activity of the three isotopes

Isotopes	Count rate with errors(cps)	Fractional intensity	Absolute activity with errors(Bq)
^{239}Pu	4.16	0.73	1652.38±40.6
^{241}Am	2.78	0.86	936.38±30.6
^{244}Cm	1.78	0.73	706.8±26.5

The MCA was calibrated at lower energies, which depend on the initial energy pulser where it was injected into the MCA and the known attenuation of the pulser output.

Table (2): shows the calibration of the MCA at low energies. The pulser output versus reference energy is plotted in figure3.

Attenuation Pulser out-put	Reference Energy(KeV)
0.5	2743
0.2	1097.2
0.14	783.7

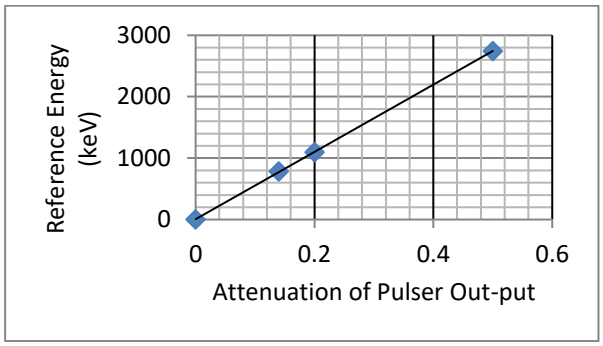

Figure (4): Calibration of the MCA via the Known pulser peaks

The FWHM is statistically calculated from equation 3. The ^{241}Am isotope is selected to present other isotopes.

The $FWHM_{total}$ of the ^{241}Am was 13.65KeV and the pulse peak ($FWHMelec_{)}$ was 7.74KeV. The value of $FWHM_{stat}$ was roughly 11.2±3.35 KeV. It could be seen that two peaks were almost close to each other.
Fiqure 5 plots the energy of three isotopes versus air pressure.

Table (3): The data of isotopes energies with pressure air

	Isotopes energy(kev)		
Pressure	^{239}Pu	^{241}Am	^{244}Cm
200	6661.96	7453.02	8239.44
400	6095.71	6860.13	7661.27
600	5584.27	6217.6	6982.85
800	4947.44	5746.56	6507.11
1000	4382.52	5125.89	5899.92

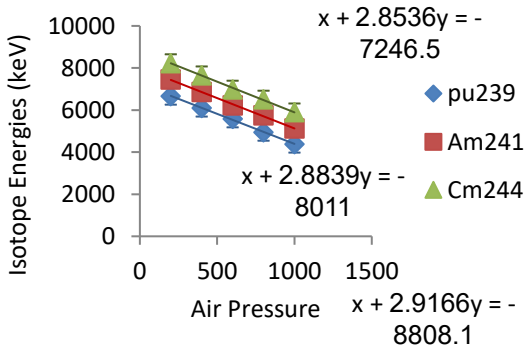

Figure (5): displays the energy of the three isotopes versus air pressure.

The equation used to calculate the extrapolated alpha particle range was:
Rα = air thickness × source to detector distance
Where Rα: is the extrapolated range of alpha particles expressed in mg/cm^2.

The source-to-detector distance is 1.5 cm, and STP is the density of the air at 1.293 mg/cm^2.

Table (4): the extrapolated range of alpha particles for the three isotopes.

Isotopes	Estimated pressure (m-bar)	Air thickness	Extrapolated range Rα cm
^{239}Pu	2539.4	3.3	4.9±2.2
^{241}Am	2777.8	3.6	5.4±2.4
^{244}Cm	3019.9	3.9	5.9±2.4

The predicted range of the same isotopes is calculated by using empirical equation 2.

Table (5): shows the predicted range of alpha particles for the three isotopes.

Isotopes	Energy of α-particles MeV	Rα cm
^{244}Cm	5.805	4.5
^{241}Am	5.486	4.1
^{239}Pu	5.155	4.5

This time, equation 4 was used to calculate the values of air thickness in order to obtain the relationship between FWHM and a small attenuation of air thickness in different positions of air pressure.

Table (6): gives data of the calculated air thickness.

	FWHM(keV)		
Air pressure (m-bar)	^{241}Am	$\Delta x^{0.5}$ mg.cm^{-2}	$\sqrt{\Delta x}$ mg.cm^{-2}
200	13.09	0.314663	0.56
400	55.2	1.326923	1.15
600	109.6	2.634615	1.62
800	67.63	1.625721	1.275
1000	66.71	1.603606	1.266

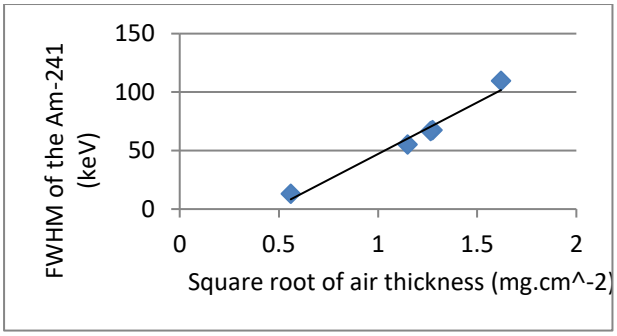

Figure (6): illustrates the direct relationship between the FWHM of ^{241}Am and the square root of the air thickness. Figure 7 illustrates the intensity of alpha particles by the count rate versus air pressure.

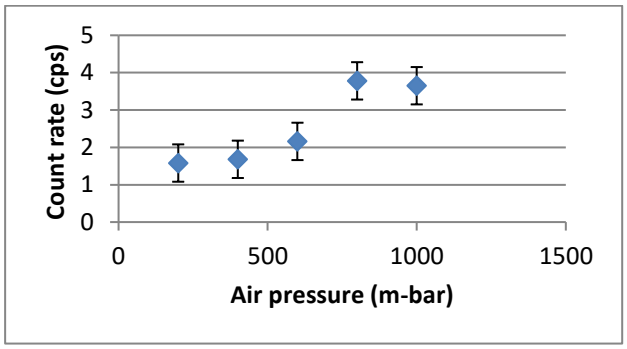

Figure (7): illustrates the intensity of alpha particles by the count rate versus air pressure.

5 Dissection

Figure 2 displays the spectrum, which has three main peaks that are relevant to the energies of the three isotopes ^{239}Pu, ^{241}Am and ^{244}Cm. The MCA calibrated the spectrum by using three dominant energies, which were 5155 keV, 5486 keV, and 5805 keV.

Figure 3 shows the inverse correlation between the dominant energy peak and the decay of the FWHM.

Figure 4 showed the correlation between the reference energy and the attenuation of pulser output. It confirms that the calibration of MCA was accurate at low energies, and that is principally due to the straggling of alpha particles.

Figure 5 illustrates the inverse relationship between the decrease in the energy of alpha particles and air pressure. The air pressure increases as the energies decrease, and this may result in the loss of energy and subsequently increase the probability of straggling alpha particles, which increase with matter in their traveling path.

The extrapolated alpha particle range is calculated via equation 3. The latter confirms the fact that the maximum alpha particle range is linked to the higher energy contained.

Figure 6 illustrates the direct correlation between the FWHM of the three isotope energy peaks and the square root of the air path thickness. It means that the probability that may occur with a small scattering of alpha particles may also be repeated in a small unit of air pressure. However, the probability of noise may increase because the straggling of alpha particles has increased.

Finally, figure 7 shows the inverse correlation between air pressure and the count rate of alpha particles, which results in the α-particles moving in a straight line with small fluctuations in energy due to their heavy mass.

6 Conclusion

The purpose of this experiment was to understand the properties of alpha particles and their interactions. The logarithmic spectrum was identified with three isotopes main energy peaks. The MCA was calibrated in order to correct the weak structures within the MCA scale at lower energies. These weak signals confirmed the straggling of alpha particles and the creation of noise within the scale. The absolute activities of the triple alpha source were determined, and they were 1652.38±40.6 Bq, 936.38 ±30 Bq, and 706.8±26 Bq for ^{239}Pu, ^{241}Am and ^{244}Cm respectively. Statistically, the full-width half maximum of ^{241}Am was calculated at 11.2±3.35 KeV. The extrapolated ranges were obtained from the curve that represented the energy peaks versus air pressure graph; they were 4.9±2.2, 5.4±2.4, 5.9±2.4.
In short, alpha particles are heavy and fully charged. They interact effectively with matter despite the fact that their penetration ability is low. In addition, they are usually not hazardous for outer exposure. One may conclude that when α-particles internally deposited, they could be more damaging than other particles due to the amount of energy deposited inside a small volume of tissue.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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