Antibacterial Activity and Phytochemical Investigation of *Matricaria Chamomilla* Plantand UV Spectra Analysisof Chaticenein Methanolic Extract

[*]Hamad. M .Adress [*] AdressHassan [**]Eman .I .Hassan .Eljadily

Abstract: The present study describes the phytochemical profile and antibacterial activity of flowers of*Matricariachamomilla* profusely prescribed in traditional medicine. The phytochemical investigations revealed many principles of bioactive properties in animal and human sterols, triterpens, flavonoids, saponins, tannins and alkaloids. The antibacterial activity was examined using agar well-diffusion method, using two extracts; distillated water andmethanolic against two gram positives (Staphylococcus aureus ATCC 25923 and Bacillus subtilis NCTC 8236) and two gram negatives (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853).The results showed that the methanolic extract have significant antibacterial activity. Alsothe UV spectra identify of Chaticene compound in methanolic extract of *Chamomile*.The current study supports theemployment of plant in folk medicine and recommends further microbiological and pharmacological studies as a promising sources for new antibacterial agents.

Introduction:-

Phytochemical Plant based natural constituents is derived from any a part of the plant like bark, leaves ,flowers ,roots ,fruits, seeds, then forth, and used as medicine by the human being since the past(Gordon and David, 2011). The beneficial medicinal effects of plant materials are typically the results of combinations of secondary products present within the plant.The medicinal values of plants are unique particular plant species or groups and are per this idea because the combination of secondary products in a very particular plant is taxonomically distinct (Wink,1990). Flavonoids are group of about 4000 present polyphenol compounds, found universally all told the plants (Harborne,1986). These are primarily recognized because the pigments to blame for the coloursof leaves, especially in autumn.

Flavonoids are cosmopolitan in fruits, vegetables, nuts, seeds, herbs, spices, stems, and flowers furthermore as tea and wine. Flavonoids are important for kinsfolk because of their antioxidative and radical scavenging effects still as their potential as estrogenic and anticancer activities (. Springob.K and. Saito . K;2002).

Chamomile (*Matricariachamomilla*) which belonging to *Asteraceae*, is one among the foremost ancient medicinal herbs known to mankind (Fatouma*et al*., 2011). Chamomile flowers are taken as herbal tea, the flowers contains 1-2% volatile oils,and rich in flavonoids (Lemberkovics*et al*., 1998). Chamomile has also numerous applications in traditional medicine; It is used as anti-cold (Saller*et al*.,1990), for gastrointestinal and digestive disorders (Kroll and Cordes, 2006), against Eczema (Nissen*et al*., 1988), anti-estrogenic effect (Kassi*et al*., 2004), anti-diabetic (Kato *et al*., 2008), for wound healing (Martins *et al*., 2009) and as an anticancer (Way *et al*., 2004).

Materials and Methods:-

[*] **Chemistry Department, Faculty of Science , Omar Al-Mukhtar University**
hamad.dr@omu.edu.ly
[*]**Chemistry Department, Faculty of Science , Omar Al-Mukhtar University**
[*] [*]**Chemistry Department , Faculty of Arts and Science , Omar Al- Mukhtar University**
eman.ibrahim@omu.edu.ly

Collection of Samples and Preparation of the Extracts:-

The spice (Chamomile) wascollected fromAl-bida City markets, Libya.The Extraction was carried out according to the method described by (Sukhdev*et al*; 2008). Briefly, 10 gram of spice were weighed and mixed with 100 ml of two different solvents (methanol and distilled water) in a conicalflask and kept in rotatory shaker at 150 rpm for 4 hours. Then, theextract wasevaporated under reduced pressure using rotary evaporator apparatus and allowed to dry in the incubator till complete dryness.

Phytochemical Screening of Spices:-

Phytochemical screening was carried out on two extracts of plant.The following tests were performed to detect various phytochemical constituents which may be present in the studied plant extracts.

Screening for Alkaloids (Mayer's Test):-

2 ml of the extract was boiled with dilute hydrochloric acid and the mixture was filtered and to the filtrate a few drops of Mayer's reagent was added.A creamy or white colour precipitate was produced immediately, indicates the presence of alkaloids.

Screening for Carbohydrate test:-

To 1ml of extract, a1ml of Benedict's reagent was added. The mixture is heated on a boiling water bath for 2 minutes.The solution appeared green showing the presence of reducing sugar.

Screening forGlycosides (Keller Kilianin Test):-

To5ml of each extract was added with 2 ml of glacial acetic acid which was followed by the addition of few drops of ferric chloride solution and 1ml of concentrated Sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.

Screening forTerpanoids (Salkowski Test):-

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3ml of concentrated sulphuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpanoids.

Screening for Flavonoids (Alkaline Reagent Test):-

2 ml of extracts was treated with few drops of 20% sodium hydroxide solution.Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Screening for Saponins (Foam Test):-

2ml of extract was taken in a test tube and 6ml of distilled water was added to it. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Screening for Steroids:-

1ml of extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

Screening for Tannins:-

2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.

Preparation of bacterial cultures

One ml aliquots of 24 h broth culture of testing organisms were aseptically added to nutrient agar slopes and incubated at 37°C for 24 h. The tested bacteria were two gram positives (Staphylococcus aureus ATCC 25923, Bacillus subtilis NCTC 8236) and two gram negatives (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853). The bacterial growth was harvested and washed off by the addition of sterile normal saline. The harvested bacteria were suspended in a suitable volume of normal saline to prepare a suspension containing about 108 -109 colony forming units per ml (CFU\ml). The suspension was stored in the refrigerator at 4 °C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra; 1938).

Antibacterial activity

Utilizing a sterile cotton swab, the suspensions of the chose bacterial strains were swabbed on the surface of sterileMueler-Hiton Agar agar plates. Agar wells were readied with the assistance of disinfected cork borer number 4, utilizing a micropipette, 100 microliters of diverse concentrations of the plant extracts (100%,75%, half, 25%)were added to different numbered wells in the plate. The plates were incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones was measured in µm and the outcomes were recorded.

Results and Discussion :-

The presence of active phytochemical constituents are the principal reasons for a plant to exhibit medicinal activity.The phytochemical analysis of Chamomileis presented in Table(1) ~~reveals.The~~Some of the phytochemical analysed were present in extracts of plant.On the eight phytochemicalsscreened alkalioid and charbohydrats and cardiacglycoside and flavonoide and saponin and thanin were presented commonly in the studies plant.In aqueousextract steroid and terpinoidswere absent~~no present~~.Differences in geographical areas may lead to variation in the chemical contents of the plant.

Table1:-The result of phytochemical screening

Plant specie	Type of extract	Metabolite							
		1	2	3	4	5	6	7	8
Matricaria Chamomilla	DW	+	++	++	++	-	-	++	+
	Methanol	+	++	++	++	++	++	++	+

Key :-1= Flavonoids 2= Alkaloids , 3= Charbohydrats , 4= Cardiac glycosides , 5= Steroid , 6= Terpenoids , 7= Saponins , 8= Tanins

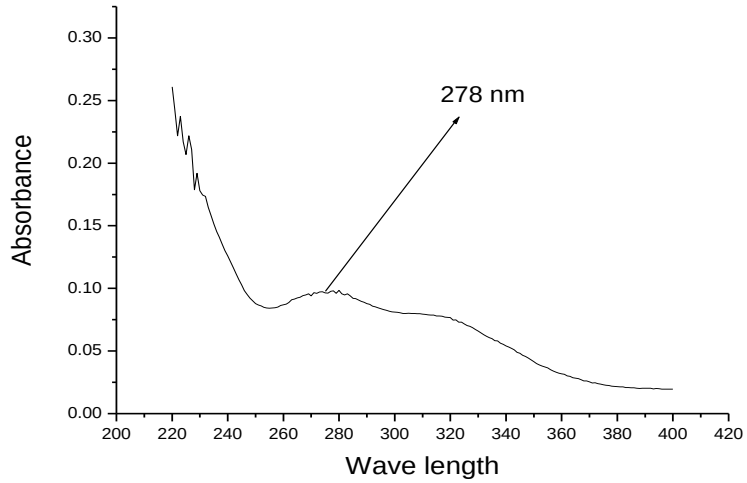
Catechin:- isaflavan-3-ol ,(2*R*,3*S*)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triola type of natural phenol and antioxidant. It is a plantsecondary metabolite. It belongs to the group of flavan-3-ols (or simply flavanols), part of the chemical family of flavonoids.

Catechin is present in many dietary products, plants, fruits (such as apples, blueberries, gooseberries, grape seeds, kiwi, strawberries), green tea, red wine, beer, cacao liquor, chocolate, cocoa, etc. The antioxidant action of catechin is well-established by various *in vitro*, *in vivo* and physical methods. Catechin affects the molecular mechanisms involved in angiogenesis, extracellular matrix degradation, the regulation of cell death, and multidrug

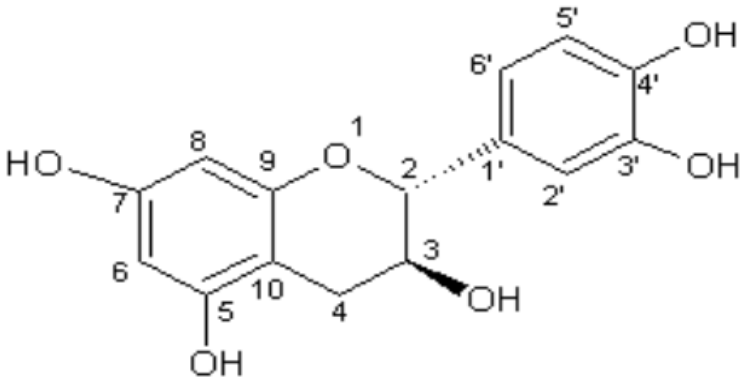
resistance in cancers and related disorders. Clinical studies have shown the beneficial effects of catechin due its antioxidant action.*(*Anand A. Zanwar, 2014).

Catechin was isolated from chamomill by using methanol. After isolation, identification of the product was done by UV visible spectrophotometer Analysis .

UV spectrophotometric method:-The UV method for the estimation of Catechinin methanol shows avalidated peaks at 278nm (Figure1).



Figuer(1): The λ max of Catechin



Figuer(2):Structure of Catechin

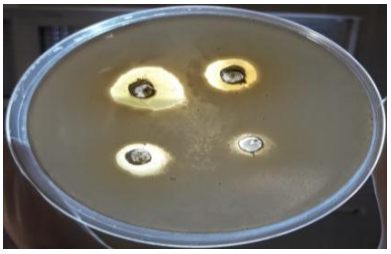
The presence of antibacterial substances within the higher plants is well established (Srinivasan, 2001). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicineis used for the treatment of diseases, or it may be the bottom for the event of a medication, a natural blueprint for the event of a drug (Didry*et al*., 1998). Successive isolation of botanical compounds from material is essentially enthusiastic about the kind of solvent utilized in the extraction procedure. The traditional healers use primarily water because the solvent but we found during, this study the plant extracts by methanol provided more consistent antimicrobial activity compared to those extracted by water. The results of antibacterial activity of *chamomille* against the investigated bacterial strains are shown in Table (2)and Figures(3,4,5,6). None of the aqueous extracts produced zones of inhibition.

This might need resulted from the shortage of solubility of the active constituents in aqueous solutions while methanol extract showed some degree of antibacterial activity. Further trials using solvents of assorted polarities will explore the consequencesof solvent composition bioactive constituents on extract efficacy (Romero *et al*., 2005). However, negative results don't mean absence of neither is that the plant inactive. Active compound(s) is also present in insufficient quantities within the crude extracts to indicate activity with the dose levels employed (Taylor *et al*., 2001). Lack of activity can thus only be proven by using large doses (Farnsworth, 1993).

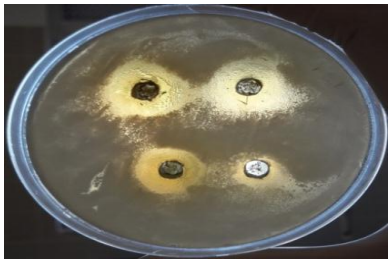
Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents (Jager*et al*., 1996). With no antibacterial activity, extracts may be active against other bacterial species which were not tested (Shale *et al*., 1999).

Table(2):Antibacterial activity of aqueous and methanol extracts of screened plant

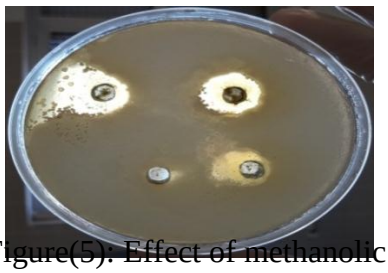
Type of bacteria	Extracts	Zone of inhibition (in µm)			
		100%	75%	50%	25%
Staphylococcus aureus	Aq	-	-	-	-
	Met	8	6	5	4
Bacillus subtilis	Aq	-	-	-	-
	Me	15	12	10	4
Pseudomonas aeruginosa	Aq	-	-	-	-
	Me	15	10	3	-
(Escherichia coli	Aq	-	-	-	-
	Me	11	9	6	3



Figure(3): Effect of methanolic extraction of *Chammomilla*against E.coli



Figure(4):Effect of methanolic extraction of *Chammomilla* against .Bacilli



Figure(5): Effect of methanolic extraction of *Chamomilla* against .Pusedo

Figure(6): Effect of methanolic extraction of *Chammomilla*against Staph .

Conclusion:-

Bacterial resistance against antibiotics is a great challenge. The results of this study showed that *Chamomile* flowers extracts have antibacterial activity against most common

bacterial strains involved in human pathogenesis. In addition,Polyphenols are one of the most numerous and diverse group of secondary metabolites; their antioxidant properties provide the basis for antimicrobial effects. The ability of some plant secondary metabolites to act as resistance-modifying agents is a promising field in mitigating the spread of bacterial resistance.

المستخلص: حيث استخدمت أزهار النبات وتم الحصول على مستخلصين *Matricariachamomilla* توضح الدراسة فعالية نبات البابونج Bacillus subtilis,Escherichia باستخدام الماء المقطر والميثانول ، وتم اختبارهما على أربع أنواع من البكتيريا الممرضة، وتنوعت نتائج دراسة الفعالية التثبيطية للمستخلصات باختلاف نوع المستخلص coli,Pseudomonas aeruginos,aureusStaphylococcus واختلاف البكتيريا المختبرة، حيث كان المستخلص الميثانولي هو الاكفأ في تثبيط جميع انواع البكتيريا.وأظهرت نتائج الكشف الفيتوكيميائي احتواء النبات على العديد من المركبات الفعالة مثل الفلافونويداتوالقلويداتوالتانيناتوالصابونيناتوالتربينات. كذلك تم تعريف مركب الكاتيكين باستخدام أطياف UV.في المستخلص الميثانولي للنبات

Key Words: Antibacterial, *MatricariaChamomilla*, Phytochemical analysis, Chaticene.

References:-

Anand A. Zanwar, Subhash L. Bodhanka .(2014)in Polyphenols in Human Health and Disease.

Didry, N., Dubreuil, L., Trotin, F., Pinkas, M. (1998): Antimicrobial activity of the aerial parts of DroserapellataSmith on oral bacteria. J. Ethnopharmacol. 60: 91-96.

Farnsworth, N.R. (1993): Biological approaches to the screening and evaluation of natural products. In: Rasoanaivo P, Ratsimamanga-Urverg S (Eds) Biological Evaluation of Plants with Reference to the Malagasy Flora, Madagascar, pp. 35-43.

Fatouma, M., Abdoul-Latif, N.M., Prosper, E.P., Adwa, A.A., Samatar, O.D., Louis-Clément, O., et al.(2011): Antimicrobial and antioxidant activities of essential oil and methanol extract of Matricariachamomilla L. from Djibouti. J. Med. Plants Res. 5(9): 1512-1517.

Gordon M.C ;David, J. N. (2001) : Natural product drug discovery in the next millennium, *Pharmaceutical Biology*,vol.39,pp.8– 17.

Harborne,J.B. (1986) : Nature, distribution and function of plant flavonoids. *Progress in Clinical and Biological Research*,vol.213, pp.15–24.

Jager, A.K., Hutchings, A., van Staden, J. (1996): Screening of Zulu medicinal plants for prostaglandinsynthesis inhibitors. J. Ethnopharmacol. 52: 95-100.

Kassi, E., Papoutsi, Z., Fokialakis, N., Messari, I., Mitakou, S., Moutsatsou, P. (2004).: Greek plant extracts exhibit selective estrogen receptor modulator (SERM)-like properties. J. Agricult.Food Chem. 52: 6956-6961.

Kato, A., Minoshima, Y., Yamamoto, J., Adachi, I., Watson, A.A., Nash, R. J. (2008): Protective effects of dietary chamomile tea on diabetic complications. J. Agricult. Food Chem. 56: 8206-8211.

Kroll, U.,Cordes, C. (2006): Pharmaceutical prerequisites for a multi-target therapy. Phytomedicine 13(5): 12-19

Lemberkovics, E., Kery, A., Marczal, G., Simandi, B., Szoke, E. (1998): Phytochemical evaluation of essential oils, medicinal plants and their preparations. ActaPharma Hung. 68(3): 109-141.

Martins, M.D., Marques, M.M., Bussadori, S.K., Martins, M.A., Pavesi, V.C., Mesquita-Ferrari, R.A., Fernandes, K.P. (2009): Comparative analysis between Chamomillarecutita and corticosteroids on wound healing : An in vitro and in vivo study. Phytother. Res. 23(2): 274-278.

Miles, A.A., Misra, S.S. (1938): The estimation of the bactericidal power of the blood. J. Hygiene, 38: 732-749.

Nissen, H.P., Biltz, H., Kreysel, H.W. (1988): Profilometry, a method for the assessment of the therapeutic effectiveness of Kamillosan ointment. Z Hautkr. 63(3):184-190.

Romero, C.D., Chopin, S.F., Buck, G., Martinez, E., Garcia, M., Bixby, L. (2005): Antibacterial properties of common herbal remedies of the southwest. J. Ethnopharmacol. 99: 253-257.

Saller, R., Beschorner, M., Hellenbrecht, D., Bühring, M. (1990): Dose-dependency of symptomatic relief of complaints by chamomile steam inhalation in patients with common cold. Eur. J. Pharmacol. 183: 728-729.

Shale, T.L., Strik, W.A., van Staden, J. (1999): Screening of plants used by southern African traditional healers in the treatment of dysmenorrhoea for prostaglandin-synthesis inhibitors and uterine relaxing activity. J. Ethnopharmacol. 64: 9-14.

Springob.K; Saito,K.(2002) : Metabolic engineering of plant secondary metabolism: promising approach to the production of pharmaceuticals."*Science and Culture*,vol.68,pp.76–85.

Srinivasan, D., Nathan, S., Suresh, T., Perumalsamy, O. (2001):Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J. Ethnopharmacol. 74:217-220.

Sukhadev, S.H., Suman, P.S.K., Gennaro, L., Dev, D.R. (2008): An overview of extraction techniques for medicinal and aromatic plants. In: Sukhdev SH et al. (Eds). Extraction technologies for medicinal and aromatic plants. International Center for Science and High Technology. Trieste, Italy.

Taylor, J.L.S., Rabe, T., McGraw, L.J., Jager, A.K., van Staden, J. (2001):Towards the scientific validation of traditional medicinal plants. Plant Growth Regul. 34: 23-37.

Way, T.D., Kao, M.C., Lin, J.K. (2004): Apigenin induces apoptosis through proteasomal degradation of HER2/neu in HER2/neu-over-expressing breast cancer cells via the phosphatidylinositol-3'-kinase/Akt-dependent pathway. J. Biol. Chem. 279: 4479-4489.

Wink, M .(1999) : Introduction: biochemistry, role and biotechnology of secondary products," *inBiochemistry of Secondary Product Metabolism*, M. Wink, Ed., pp. 1–16, CRC Press, Boca Ratom, Fla, USA.