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Phytochemical Screening and Antibacterial Activity of Aqueous and Ethanolic Extracts from *Marrubium Vulgare* L Plant

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A B S T R A C T

Marrubium vulgare L., a remarkable medicinal herb, has been used for centuries to capably treat various ailments, including coughs, colds, and digestive issues. Renowned for its expectorant, diaphoretic, and anti-inflammatory properties, this precious herb has become a cornerstone of traditional medicine worldwide. In this study, the productivity of the ethanolic extract was an impressive 33.69%, while the aqueous extract showcased a still remarkable 14.5%. The pH levels confirmed the ethanolic extract at 4.02 and the aqueous extract at. 6.61. Phytochemical screening exposed the existence of beneficial compounds, such as alkaloids, flavonoids, saponins, phenols, steroids, and terpenes in both extracts. It is noteworthy that the ethanolic extract exhibited a higher presence of tannins, which were not found in the aqueous extract. In contrast, the ethanolic extract did not contain phenols, whereas the aqueous extract contained a significant amount of these compounds. To further explore the biological potential of these extracts, this study was conducted on their activity against *E. coli* and *S. aureus*. The findings revealed that the aqueous extract's astounding inhibition zones, measured at 19 mm and 17 mm against *E. coli* and *S. aureus,* respectively. The ethanolic extract also exhibited noteworthy results, with inhibition zones of 16 mm and 18 mm against *E. coli* and *S. aureus* correspondingly. Clearly, *Marrubium vulgare* L. is a truly remarkable herb with immense medicinal value.

1. Introduction

Plants play a vital role in their environment, making them incredibly valuable to humans and animals. They provide many benefits and produce various components that aid in their growth and protection. These plants are categorized as medicinal, food, or aromatic plants because they provide a multitude of benefits to the environment in which they flourish. Among these plants is *Marrubium vulgare* L, where this plant is an extraordinary plant that holds great promise in the realm of biomedicine. It boasts an abundance of bioactive compounds, including the exclusive diterpene marrubiin, found only in plants of this genus. Also, this notable herb contains an intricate blend of phenolic compounds. Countless studies have unveiled the impressive antioxidant properties of *Marrubium vulgare* L, unlocking its potential as a formidable ally in the fight against cancer, diabetes mellitus, and liver diseases. *Marrubium vulgare* L. has its origins in the region spanning from the Mediterranean Sea to Central Asia, has undergone significant adaptation to a globally distributed species found on every continent (Knoss, 1999). The most widely known folk name in the Libyan accent for this herb is "Robia", historically used by women as a tea for its bitter remedial properties when struggling with infertility or to regulate the menstrual cycle. Nevertheless, *Marrubium vulgare* L is globally renowned for its traditional use in treating gastrointestinal and respiratory disorders. *Marrubium vulgare* L. is an herb from the *Lamiaceae* family with a tough, woody taproot and erect, downy stems. Leaves are roundish, toothed, veined, and hoary, the inflorescence showcases an array of striking white flowers that are densely arranged in axillary

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whorls, and Calyx is tubular, lobed, and 10-toothed, with a small hooked spine or bristle (Yabrir, 2019). The report indicates the effective extraction of phytoconstituents from this plant. The alcohol-soluble extractive value is 8.66%, suggesting high solubility. The estimated water-soluble extractive value is 5.90%, while the petroleum ether-soluble extractive value is 2.77% (Mittal, 2016). In addition, *Marrubium vulgare* L. is a rich source of a wide variety of phenolic compounds, including major classes such as phenolic acids, phenyl propanoid (cinnamic) acids, esters, and flavonoids (Boudjelal, Henchiri, Siracusa, Sari, & Ruberto, 2012). The aim of this study is to identify certain secondary metabolites and assess their potential as an antidote against pathogenic bacteria that affect humans.

2. Materials and Methods

2.1 Harvesting and processing the plant for extraction: The *Marrubium Vulgare* L plant was carefully collected from the Alkhums region, situated approximately 120 kilometers east of Tripoli, the capital city of Libya, during its thriving spring season in 2022. The process involved meticulous steps, from gathering healthy leaves to washing them with both tap and distilled water, followed by a thorough drying process in the shade and then in an electric oven at 43 degrees Celsius. To gain a fine powder from the plant leaves, the dried leaves were ground with an electric mixer and sieved with a 500-micron sieve, before being stored in tightly sealed, opaque glass bottles in a dry place until further use.

2.2 Extraction: During the extraction and separation stage, the aqueous-ethanolic extract method was employed. To begin with, 20g of finely powdered plant leaves were accurately measured and placed in a 500-ml conical flask. Subsequently, 400 ml of the suitable solvent, distilled water, and ethyl alcohol were added separately. To ensure thorough extraction, the flasks were securely capped and placed on a shaker at 100 rpm for 72 hr. Following this, the mixture was carefully filtered, revealing a brown color for the water extract and an enticing, oily green shade for the alcoholic extract.

2.3 Productivity Calculation: Following filtration, a rotary evaporator efficiently separated the solvent from the raw material at 40 °C. The dry aqueous extract exhibited a rich brown hue, while the alcoholic extract presented an alluring, oily green tint. The productivity percentage was determined using the following formula:

$$\textit{Productivity\%} = (\textit{weight of dry extract} \div \textit{weight of fine leave powder}) \times 100 \quad (1)$$

2.4 Measuring pH levels: A 100-ml beaker contained 1g of dry crude extracts of *Marrubium Vulgare* L (aqueous and ethanolic, separately) dissolved in 10 ml of distilled water at 25 ±1 °C. The pH meter electrode from Hanna Instruments was carefully inserted into the beaker, providing the readings displayed in Table 1.

2.5 Phytochemical Screening

2.5.1 Detecting qualitative phytoconstituents in plant crude extracts: The crude aqueous and ethanolic extracts were subjected to a qualitative analysis of secondary metabolites. The analysis followed the procedures outlined by (Harborne, 1973), (Edrah S. , 2021), (Agency, 2012) (Amessis-Ouchemoukh, 2014), (Uddin, 2011a, 2012b), with some modifications. The purpose of the analysis was to identify which extract contained the metabolites. The crude extracts were tested for various secondary metabolites.

2.5.1.1 Detection of alkaloids: To identify alkaloids, extract 2 ml of the crude (aqueous, alcoholic, separately) and placed it in a test tube. Next, add 6 ml of 2N HCl (Hydrochloric Acid) and bring to a boil one minute. Then, a volume of 1 ml of Wagner's reagent was added. The presence of alkaloids in the plant will be indicated by a mesmerizing reddish-brown color with the formation of a precipitate.

2.5.1.2 Detection of flavonoids: 5 ml of the crude extract (aqueous-alcoholic separately) is placed in a test tube, and 3 to 4 drops of 0.05 NaOH solution (Sodium Hydroxide) are added to it. The yellow color that changes to colorless after adding 3-4 drops of 0.1% HCl serves as evidence of the presence of flavonoids in the plant extract.

2.5.1.3 Detection of saponnins

A. **Foam Test:** 5 ml of the crude aqueous-alcoholic extract separately and place it in a test tube. Shake vigorously for 3 minutes. The formation of foam will indicate the presence of saponnins.
B. **Frothing test:** Place 5 ml of the crude (aqueous-alcoholic, separately) into a test tube, add 3 drops of olive oil, and thoroughly shake for 3 minutes. It will be a suspension of butter pieces or an emulsion, evidence of the presence of saponnins in the sample.

2.5.1.4 Detection of tannins and phenols: (Ferric Chloride test, $FeCl_3$): 5 ml of the crude (aqueous-alcoholic, separately) and then 2 to 3 drops of $FeCl_3$ solution were added. The presence of tannins and phenols is indicated by the development of dark green, dark blue, or black color.

2.5.1.5 Detection of steroids and terpenes: 3 ml of the crude (aqueous, alcoholic, separately) were mixed with 5 ml of chloroform. After thorough mixing, a few drops of concentrated HCl were slowly added. The appearance of a reddish-brown color with a ring between the two phases indicates the presence of steroids. A very dark red color indicates the presence of terpenes.

2.5.1.6 Detection of glycosides: To detect the presence of glycosides, mix 10 ml of the crude extract with 4 drops of concentrated HCl. Next, add 3 drops of NaOH solution, followed by 3–4 drops of Fehling's solution. Finally, heat the reaction mixture and observe the formation of a red precipitate.

2.5.1.7 Detection of reducing sugars: To detect the presence of reducing sugars in the crude extract, 10 ml of extracts were mixed with 1 ml of distilled water. After vigorous shaking and careful filtration, 5–6 drops of Fehling's solution were added to the mixture. Boiling this enhanced mixture for

2 minutes led to the striking formation of an orange-red precipitate.

2.5.1.8 Detection of anthraquinones: Take 10 mL of the crude extract and transfer it into a test tube, then, add 3–4 drops of HCl (10%), ensuring a perfect mixture. Once this is done, filtrate the reaction mixture and let it cool at room temperature. Then, carefully combine the filtrate with an equivalent volume of Chloroform ($CHCl_3$). Next, add 3 drops of NH_3 aqueous solution (10%) and gently heat the filtrate. If the solution transforms into a captivating rose-pink hue, it unequivocally confirms the existence of anthraquinones.

2.5.1.9 Detections of amino acids, and proteins: To 10 ml of the crude extract, 3 drops of Ninhydrin solution $C_6H_4(CO)_2C(OH)_2$ (0.2%) were added and heated in a water bath for 5 minutes. The appearance of the blue color indicates positive results for amino acids and proteins.

2.5.1.10 Detection of cardiac glycosides: To 10 ml of the crude extract, 0.5 ml of Glacial Acetic Acid *(*$C_2H_4O_2$*)* was added. The mixture was supplemented with 4 drops of $FeCl_3$ solution $FeCl_3$ (5.0%) and 3 drops of concentrated H_2SO_4. The appearance of a greenish-blue colour confirmed the presence of cardiac glycosides.

2.6 Assessment of Antibacterial Activity

This research utilized two distinct types of bacteria: the gram-negative *Escherichia coli* (*E. coli*) and the gram-positive *Staphylococcus aureus* (*S. aureus*). These bacterial strains were carefully isolated from Alkhums Teaching Hospital in Alkhums, Libya. To cultivate the bacteria, the cultures underwent a meticulous incubation process for a duration of 24 hours, maintaining a precise temperature of 37 °C. The bacterial activity of the aqueous and alcoholic extracts of *Marrubium Vulgare* L leaves will be analyzed using the disk diffusion method in this study (Bauer, 1966).

2.6.1 Disc Diffusion Method: The disk diffusion technique for evaluating antimicrobial susceptibility was conducted under the standard protocols established by Bauer in 1966. This method was employed to evaluate the antibacterial effectiveness of both aqueous and alcoholic extracts derived from plants. Bacterial cultures were adjusted to the 0.5 McFarland standard and evenly inoculated onto Mueller-Hinton agar plates using a sterile swab. After a drying for 15 minutes, the plates were readied for sensitivity testing. Discs measuring 6 mm in diameter and impregnated with the respective extracts were placed on the agar surface. Also, two positive control antibiotics were incorporated: 30 µg of Ampicillin for *Staphylococcus aureus* and 30 µg of Ciprofloxacin for *Escherichia coli*. The agar plates were subsequently incubated at 37 °C for 24 hours, according to the specific bacterial strain being tested. After incubation, the diameters of the zones of inhibition were measured in millimeters. To ensure the reliability of the findings, the experiment was repeated three times, with the results summarized in Table 3.

3. Results and Discussion

3.1 Results of productivity and pH estimation

Table 1 Results of productivity and pH estimation

Extract Types	Yield (%)	pH
Aqueous	14.5	6.61
Ethanolic	33.69	4.02

According to Table 1, the productivity of ethanolic extract (33.69%) significantly surpassed that of the aqueous extract (14.5%). Furthermore, previous studies (500, 501) identified 34 components in the oil, accounting for 95.1% of the total oil. The essential oil stands out for its high concentration of sesquiterpenes (82.5%), with beta-bisabolene (25.4%), beta-caryophyllene (11.6%), and E-beta-farnesene (8.3%) as the primary components (JS., 2002), (D., 2003). Moreover, the pH level of the ethanolic extract (4.02) was notably lower than that of the aqueous extract (6.61).

3.2 Phytochemical Screening, detecting qualitative phytoconstituents in plant extracts

Table 2 Results of detecting qualitative phytoconstituents in plant extracts

Constituents	Extracts Types	
	Aqueous	**Ethanolic**
Alkaloids	+++	+
Flavonoids	+++	++
Saponnins	+++	+++
Tannins	-	+++
Phenols	+++	-
Steroids and Terpenes	+++	+++
Glycosides	++	+
Reducing Sugars	++	+
Anthraquinones	+	+
Amino Acids & Proteins	+	+
Cardiac Glycosides	+++	++

Key: +++ indicates abundance, ++ indicates moderate quantity, + indicates low quantity, and - indicates absence.

Table 2 reveals several qualitative phytoconstituents in both aqueous and ethanolic plant extracts, such as alkaloids, flavonoids, saponnins, phenols, steroids, terpenes, glycosides, reducing sugars, anthraquinones, amino acids and proteins, and cardiac glycosides. While tannins were not exitance in the aqueous extract, they were found in considerable quantities in the ethanolic extract. Else, the aqueous extract exhibited an vital presence of phenols, which, interestingly, were absent from the ethanolic extract. *Marrubium vulgare* L contains flavonoids, coumarin, xanthone, nitrogen compounds, amino acids, alkaloids, polysaccharides, and minerals, signifying its potential health benefits (Uddin, 2011a, 2012b), (Paunovic, 2016), (Verma & al, 2012), (Kurbatova, 2003). It also holds components such as tannins and saponnins, which may be responsible for its antibacterial effectiveness. Also, the existence of phenols in the aqueous extract of *Marrubium vulgare* L

places of interest its potential antioxidant activity, which could contribute to its health benefits. The mixture of phytoconstituents originate in this plant, including alkaloids, flavonoids, saponnins, tannins, and phenols, proposes a complex chemical profile that may proposition a extensive range of curative properties. The complex chemical profile of *Marrubium vulgare* L make available a source for its potential beneficial properties, as alkaloids, flavonoids, saponins, tannins, and phenols all contribute to its variety of health assistances. Though, further research is obligatory for plentifully understand the mechanisms behind these possessions and discover its potential applications in medicine.

3.2 Antibacterial Activity:

Table 3: Results of the *Marrubium Vulgare* L aqueous and ethanolic extracts activity against bacteria:

Bacterial Types	Extracts inhibitory zones (mm)		Antibiotic (mm)	
	Aqus	EtOH	Ampi	Cipro
E. coli	19	16	24	19
S. aureus	17	18	23	22

Key: Aqus = Aqueous, EtOH = Ethanolic, Ampi = Ampicillin, Cipro = Ciprofloxacin.

Table 3 presents compelling evidence of the biological activity of two extracts from the *Marrubium vulgare* L plant against *E. coli* and *S. aureus*. The Aqueous extract displayed impressive inhibition zones of 19 and 17 mm against *E. coli* and *S. aureus*, respectively, while the ethanolic extract showed 16 and 18 mm against E. coli and S. aureus, respectively. These results strongly suggest that the compounds in the plant's leaves may have a significant impact on bacterial resistance. More research is needed to isolate and identify the precise compounds that are responsible for the antibacterial activity. Additionally, it is important to assess their potential use in pharmaceutical or antimicrobial applications. This study opens up exciting possibilities for the development of new antibacterial agents derived from natural sources. Medications made from plants are easily available, affordable, safe, effective, and rarely cause side effects. Using plants with a long history of traditional healing is the most sensible method for developing potent new therapeutic drugs, including treatments for cancer (P. M. Dewick, 1966), (Phillipson, 1966). Despite the advances made in the field of chemotherapy, the utilization of medicinal plants has recently witnessed a significant rise. This can be attributed to several reasons, including the extraction of active pharmacological compounds from these plants, which serve as valuable sources. Additionally, medicinal plants are being used as precursors for chemic-pharmaceutical synthesis. Moreover, in industrialized countries, there has been an upsurge in the use of medicinal plants for preparations of herbal medicines (R. Magherini, 1998).

1 Conclusions

Marrubium vulgare L., a medicinal plant with numerous vital, has been used for centuries to heal several diseases. In this study, the yield of the ethanolic and aqueous crude extracts was notable, with the ethanolic extract having a higher yield. Both crude extracts contained advantageous composites, but they differed in their tannin and phenol consists. The crude extracts as well displayed promising activity against *E. coli* and *S. aureus*. Overall, it can be concluded that *Marrubium vulgare* L. is a distinguished herb with great medicinal value.

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