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Investigation of Essential Minerals and Antimicrobial Effects of Selected Medicinal Herbs

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Abstract: Three medicinal plants that are often employed in traditional remedies—Phagnalon rupestre, Marrubium vulgare, and Ajuga iva—were examined for their mineral content and antibacterial activity. Plant materials were collected from Msallata region of Libya, cleaned, air-dried for 25 days, then crushed, sieved, and sealed in glass bottles. 20 g of plant powder was macerated in 200 mL of ethanol at room temperature for 72 hours to create ethanol extracts, which were then filtered, concentrated, and dried at 40°C. Using atomic absorption spectroscopy and flame photometry following wet digestion with concentrated HNO_3, mineral analysis focusses on three macroelements (Na, K, and Ca) and three microelements (Fe, Cu, and Zn). According to the results, Ajuga iva had the greatest zinc level (168.44 mg/L), whereas Marrubium vulgare had the highest sodium (850 mg/L) and iron content (4375 mg/L). All three species have high levels of calcium, ranging from 13,479 to 15,992 mg/L. Only Phagnalon rupestre had copper (147 mg/L). Selective action was shown in antimicrobial tests against Staphylococcus species and Escherichia coli. Phagnalon rupestre only demonstrated inhibition against Staphylococcus at the highest concentration, but Ajuga iva showed considerable inhibition against E. coli and Staphylococcus at 12.5–50% concentrations. There was no antibacterial activity in Marrubium vulgare. As anticipated, standard antibiotics such as amikacin, gentamicin, penicillin, and tetracycline showed bigger inhibitory zones.

Keywords: Medical plants, Antimicrobial, Metal contents, Marrubium, Ajuga, Phagnalon

Introduction:

For thousands of years, medicinal plants have been an essential component of traditional healthcare systems, and they still play a critical role in the prevention and treatment of disease all over the world. The World Health Organisation (WHO) estimates that between 70 and 80 percent of people in underdeveloped nations get their basic medical treatment mostly from plant-based medications. This extensive reliance emphasises the medicinal value of the large range of bioactive substances found naturally in plants [1-5].

Numerous secondary metabolites, such as phenolic acids, flavonoids, terpenoids, organic acids, vitamins, and vital minerals, are known to be present in plants. These substances have a variety of positive physiological benefits on humans, including antioxidant, anti-inflammatory, and antibacterial properties, in addition to helping plants defend against biotic and abiotic challenges [6-8]. Because it presents prospects for their exploitation in pharmacological, nutritional, and medical applications, the natural occurrence of such bioactive substances calls for a thorough scientific examination. Furthermore, knowing the chemical makeup of medicinal plants offers a logical foundation for the synthesis of related bioactive derivatives, which is frequently more effective than using random synthetic methods [9-11].

Many aromatic and therapeutic herbs have long been used, but their chemical and biological properties are still mostly unknown. While certain species that are not commonly utilised may have beneficial medicinal qualities, others may provide health hazards if handled incorrectly [12]. Therefore, before adding therapeutic plants to the diets of humans or animals, it is crucial to assess both their biological activity and nutritional worth. Chemical characterisation also helps with precise plant identification, lowering the possibility of incorrect identification and guaranteeing uniformity in therapeutic application [13,14].

Globally, infectious illnesses brought on by pathogenic bacteria continue to be a leading cause of morbidity and mortality. One of the most significant worldwide public health issues of the twenty-first century is the rapid development of antibiotic resistance in recent decades [15]. The hunt for substitute antimicrobial drugs has increased due to the diminished efficacy of traditional antibiotics. About 30–40% of contemporary medications are either directly or indirectly obtained from natural sources. Natural products, especially those produced from plants, have historically been significant sources of therapeutic treatments [16,17]. This has renewed interest in medicinal plants as potential reservoirs of novel antimicrobial agents with fewer side effects [18].

The current study's objectives were to determine the antibacterial activity and mineral composition (Na, K, Ca, Fe, Cu, and Zn) of ethanol extracts from Phagnalon rupestre, Marrubium vulgare, and Ajuga iva, as well as their possible nutritional and medicinal value against specific bacterial strains (Escherichia coli and Staphylococcus sp.).

Experimental part

Plant collection and preparation

In April 2025, leaf samples of Ajuga iva Schreb., Marrubium vulgare L., and Phagnalon rupestre L. were taken from the Msallata region of Libya. To get rid of adherent dust and contaminants, the gathered plant materials were carefully cleaned many times using tap water and then distilled water. After that, the samples were allowed to air dry for 25 days at room temperature. Following drying, the plant materials were pulverised in an electric grinder, sieved to produce a homogenous powder, and then kept in glass vials that were sealed for further examination [19].

The plant species were collected from the Msallata area and taxonomically identified by experts from the Department of Botany, Faculty of Arts and Science, El-Mergib University.

Extraction method

After macerating 10.0 g of each dried plant powder in 100 millilitres of ethanol, the mixture was left to remain at room temperature for 72 hours. The solvent volume was divided into three sections (30 mL, 30 mL, and 40 mL) in order to do the extraction step-by-step. To guarantee optimal extraction efficiency, the mixture was filtered using Whatman No. 1 filter paper after each extraction stage, and the plant residue was then extracted again using the subsequent solvent portion. The ethanolic extracts were not concentrated for antimicrobial tests; instead, the liquid extracts were utilised directly for antibacterial testing and kept in dark glass bottles at 4°C to avoid bioactive component degradation [20].

Mineral Content Analysis

The concentrations of selected macroelements and microelements, namely sodium (Na), potassium (K), calcium (Ca), iron (Fe), copper (Cu), and zinc (Zn), in the powdered plant samples were determined using appropriate analytical techniques. Iron (Fe), copper (Cu), and zinc (Zn) were analyzed using a Flame Atomic Absorption Spectrometer (AA-6800, Shimadzu, Japan), while sodium (Na), potassium (K), and calcium (Ca) were determined using a flame photometer (Jenway, Model Model PFP7, UK) [21].

The plant samples underwent wet digestion before analysis. After digesting a known mass of each powdered sample with strong nitric acid (HNO_3), hydrogen peroxide ($H2O_2$) was added gradually. The digesting mixture was heated gradually until the organic matter was completely oxidised and a clear solution was produced. Following cooling, the digested samples were diluted with distilled water to a predetermined volume and filtered if needed [22,23].

All glassware used in the analysis was soaked overnight in a nitric acid solution, thoroughly rinsed three times with distilled water, and dried before use to avoid metal contamination.

Antimicrobial Activity Assays

The antimicrobial activity of the ethanolic plant extracts was evaluated using the agar well diffusion method. Two bacterial strains were used as test microorganisms: Escherichia coli (Gram-negative) and Staphylococcus sp. (Gram-positive). Fresh bacterial cultures were prepared and adjusted to an appropriate turbidity before use [24].

Sterile nutrient agar plates were prepared and uniformly inoculated with the bacterial suspensions using sterile cotton swabs to ensure even distribution of the microorganisms over the agar surface. After inoculation, sterile cork borers were used to punch wells of uniform diameter in the agar medium [25].

Different concentrations of the plant extracts were prepared (100%, 50%, 25%, and 12.5%), and a fixed volume of each extract was carefully introduced into the respective wells. Control wells containing standard antibiotics were included for comparison, while solvent controls were used to confirm the absence of antimicrobial effects from the extraction solvent. The plates were then allowed to stand at room temperature for a short period to facilitate diffusion of the extracts into the agar medium [26].

Following diffusion, the inoculated plates were incubated at 37 °C for 24 hours. After incubation, the antimicrobial activity was assessed by measuring the diameter of the inhibition zones (in millimeters) surrounding each well. The absence of a clear zone was recorded as resistance (R). All experiments were conducted under aseptic conditions to avoid contamination [27].

Chemicals and reagents

All chemicals and reagents used in this study were of analytical reagent grade and were used without further purification. Ethanol (≥99.8%), hydrochloric acid (HCl, 37%), concentrated nitric acid (HNO_3, 65%), and hydrogen peroxide (H_2O_2, 30%) were obtained from Merck (Darmstadt, Germany). Double distilled water was used throughout all experimental procedures.

Certified standard stock solutions of sodium (Na), potassium (K), calcium (Ca), iron (Fe), copper (Cu), and zinc (Zn) with a concentration of 1000 mg/L were used for instrument calibration and preparation of calibration curves. The working standard solutions were prepared by appropriate dilution of the stock solutions with distilled water.

Results and discussion

Mineral Element Composition of the Studied Medicinal Plants

The mineral composition of three medicinal plants, Marrubium vulgare L., Ajuga iva Schreb. L., and Phagnalon rupestre, was determined in order to evaluate their nutritional and medicinal value. The concentrations of three macroelements (sodium, potassium, and calcium) and three microelements (iron, copper, and zinc) are presented in Table 1, while their distribution is illustrated in Figures 1 and 2.

Table 1. Concentrations of macro- and micro-elements (mg/L) in selected medicinal plants

Element	Unit	*Marrubium vulgare* L.	*Phagnalon rupestre*	*Ajuga iva* Schreb. L.
Macroelements				
Sodium (Na)	mg/L	850.0	650.0	700.0
Potassium (K)	mg/L	165.0	165.0	100.0
Calcium (Ca)	mg/L	15992.0	13479.0	15280.0
Microelements				
Iron (Fe)	mg/L	4375.0	2079.0	3688.0
Copper (Cu)	mg/L	< 2.0*	147.0	< 2.0*
Zinc (Zn)	mg/L	103.13	79.06	168.44

under detection limits

Macroelements (Na, K, Ca) contents

The results indicate that calcium was the most abundant macroelement in all studied plants, followed by sodium and potassium. Calcium concentrations ranged from 13479 mg/L in Phagnalon rupestre to 15992 mg/L in Marrubium vulgare, with an average value of approximately 14917 mg/L. The high calcium content observed in all samples highlights the potential nutritional importance of these plants, as calcium plays a crucial role in bone formation, muscle contraction, blood clotting, and enzymatic regulation.

Sodium concentrations varied from 650 mg/L in Phagnalon rupestre to 850 mg/L in Marrubium vulgare, with an average concentration of about 733 mg/L. Sodium is an essential electrolyte responsible for maintaining fluid balance, nerve impulse transmission, and muscle function. Although sodium was present at lower levels compared to calcium, its moderate concentration contributes to the overall electrolyte profile of the plants.

Potassium levels ranged between 100 mg/L in Ajuga iva and 165 mg/L in both Marrubium vulgare and Phagnalon rupestre, giving an average value of approximately 143 mg/L. Potassium is vital for maintaining normal cell function, regulating heart rhythm, and controlling blood pressure. The relatively balanced potassium content in Marrubium vulgare and Ajuga iva suggests their potential role in dietary mineral supplementation.

Overall, Marrubium vulgare showed the highest total macroelement content, particularly for calcium and sodium, while Phagnalon rupestre exhibited the lowest calcium concentration among the studied plants.

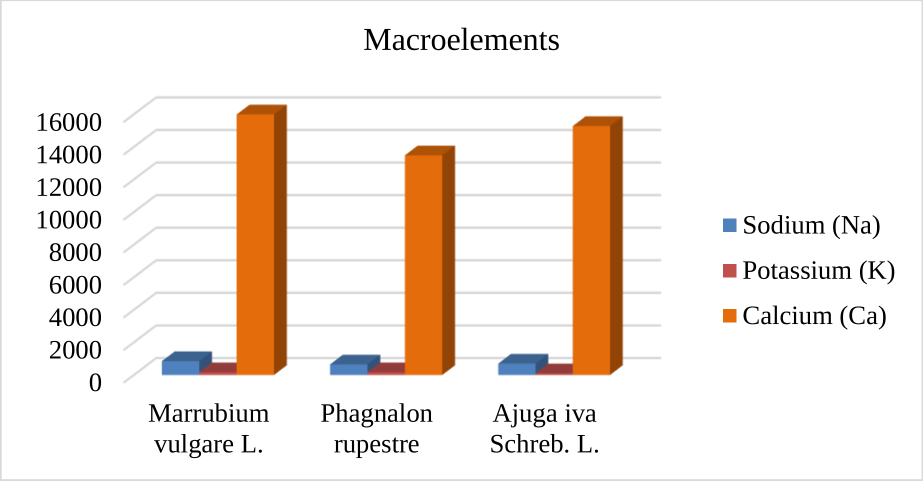

Fig. 1. Distribution of macroelement concentrations (Na, K, and Ca) in selected medicinal plants (Marrubium vulgare L., Ajuga iva Schreb. L., and Phagnalon rupestre)

Microelements (Fe, Cu, Zn)

The microelement analysis revealed that iron was the dominant trace element in all plant samples. Iron concentrations ranged from 2079 mg/L in Phagnalon rupestre to 4375 mg/L in Marrubium vulgare, with an average concentration of approximately 3381 mg/L. Iron is an essential element involved in hemoglobin synthesis, oxygen transport, and various metabolic processes. The high iron content, particularly in Marrubium vulgare and Ajuga iva, supports their traditional use in herbal medicine, especially in treatments related to anemia and fatigue.

Zinc concentrations showed noticeable variation among the plants, ranging from 79.06 mg/L in Phagnalon rupestre to 168.44 mg/L in Ajuga iva, with an average value of about 117 mg/L. Zinc is an important micronutrient involved in immune system function, protein synthesis, wound healing, and enzymatic activity. The relatively high zinc content in Ajuga iva enhances its potential nutritional and therapeutic value.

Copper was detected at 147 mg/L only in Phagnalon rupestre, while its concentration was below the detection limit (< 2.0 mg/L) in both Marrubium vulgare and Ajuga iva. Copper is required in trace amounts for iron metabolism, antioxidant defense, and connective tissue formation. The presence of copper exclusively in Phagnalon rupestre may indicate species-specific uptake or accumulation mechanisms.

In general, Marrubium vulgare exhibited the highest iron content, Ajuga iva showed the highest zinc concentration, and Phagnalon rupestre was distinguished by the presence of copper.

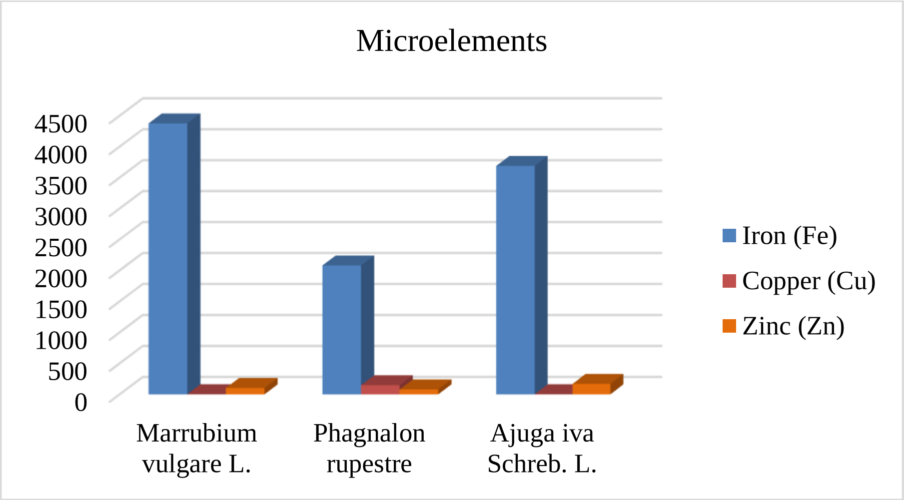

Fig. 2. Distribution of microelement concentrations (Fe, Cu, and Zn) in selected medicinal plants (Marrubium vulgare L., Ajuga iva Schreb. L., and Phagnalon rupestre)

The mineral profile observed in this study for Marrubium vulgare, Phagnalon rupestre, and Ajuga iva revealed significantly higher concentrations of certain elements compared to previous reports, particularly for Ajuga iva. For instance, our results for A. iva showed calcium (15,280 mg/L) and iron (3,688 mg/L) levels that far exceed those reported by Lahrizi et al. (2024) [6], who found calcium between 32.07–58.76 mg/L and iron between 0.61–2.29 mg/L. Similarly, the iron content in our study is notably higher than the values reported by Makni et al. (2013) [3] (2.3433 mg/g) and Senhaji et al. [28] (112 mg/L). While Ammar et al. (2022) [8] reported higher potassium levels (45,035.7 mg/kg) in A. iva leaves, our findings for sodium (700 mg/L) and zinc (168.44 mg/L) remain substantially higher than the ranges reported in recent Moroccan and Tunisian studies. These discrepancies in macro- and microelement concentrations may be attributed to differences in geographical locations, soil composition, and the specific phenological stages of the plants at the time of collection.

Antibacterial Activity of Ethanolic Plant Extracts

The antibacterial activity of ethanolic extracts of Ajuga iva, Phagnalon rupestre, and Marrubium vulgare was evaluated against Escherichia coli and Staphylococcus sp. using the agar diffusion method. The results are presented in Figures 3 and 4, while the complete numerical data are summarized in Table 2.

Table 2. Inhibition zones (mm) of ethanolic extracts of selected medicinal plants at different concentrations and standard antibiotics against Escherichia coli and Staphylococcus sp.

Test plant	Concentration (%)	Inhibition zone (mm) – *E. coli*	Inhibition zone (mm) – *Staphylococcus* sp.
Ajuga iva	100	R	R
	50	13	18
	25	12	R
	12.5	9	R
Phagnalon rupestre	100	R	12
	50	R	R
	25	R	R
	12.5	R	R
Marrubium vulgare	100	R	R
	50	R	R
	25	R	R
	12.5	R	R
Tetracycline	10 µg	25	22
Gentamicin	10 µg	20	23
Penicillin	1.5 µg	30	25
Amikacin	30 µg	24	16

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Antibacterial activity against Escherichia coli

As shown in Figure 3, E. coli exhibited a limited susceptibility to the tested plant extracts. Among the three plants, Ajuga iva demonstrated the highest antibacterial activity, producing measurable inhibition zones at 50%, 25%, and 12.5% concentrations (approximately 13, 12, and 9 mm, respectively). Interestingly, no inhibition was observed at 100% concentration, which may be attributed to poor diffusion of highly concentrated extract components through the agar medium or possible antagonistic interactions between bioactive compounds at higher concentrations [10].

In contrast, ethanolic extracts of Phagnalon rupestre and Marrubium vulgare showed no inhibitory effect against E. coli at all tested concentrations. The intrinsic resistance of Gram-negative bacteria, which possess an outer membrane rich in lipopolysaccharides that acts as an effective permeability barrier, limiting the penetration of many plant-derived antimicrobial compounds, can explain this lack of activity [24].

The antibiotic controls showed strong inhibition zones ranging from 20 to 30 mm, confirming the susceptibility of the tested E. coli strain and validating the experimental method. The markedly higher inhibition zones produced by antibiotics compared to plant extracts highlight the relatively moderate antibacterial potency of the crude ethanolic extracts.

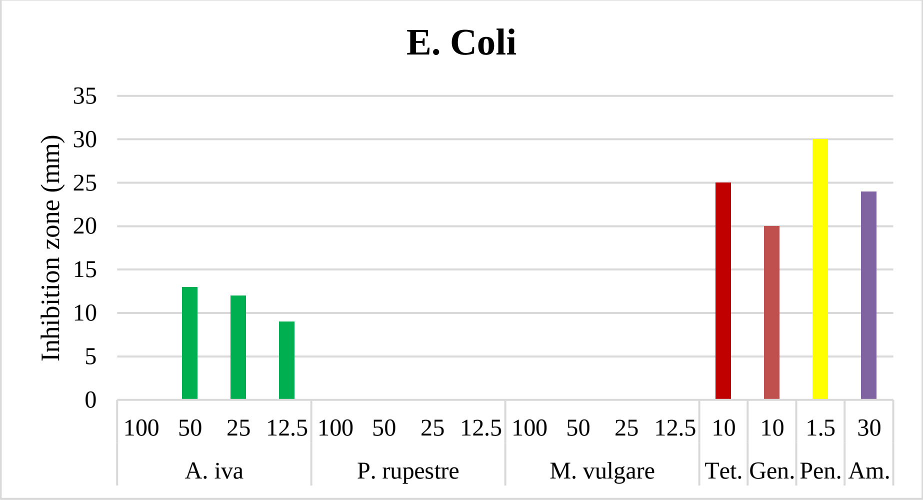

Fig. 3. Antibacterial activity of ethanolic extracts of Ajuga iva, Phagnalon rupestre, and Marrubium vulgare at different concentrations against Escherichia coli.

Antibacterial activity against Staphylococcus sp

Figure 4 illustrates that Staphylococcus sp. was more susceptible to the plant extracts than E. coli. This observation is consistent with the known structural differences between Gram-positive and Gram-negative bacteria. The absence of an outer membrane in Gram-positive bacteria facilitates the diffusion of antimicrobial phytochemicals into the bacterial cell.

The ethanolic extract of Ajuga iva exhibited pronounced antibacterial activity against Staphylococcus sp., with a maximum inhibition zone of approximately 18 mm at 50% concentration. However, no activity was detected at the other tested concentrations, suggesting that the antibacterial effect of Ajuga iva is concentration-dependent and optimal at intermediate concentrations.

Phagnalon rupestre showed weak antibacterial activity against Staphylococcus sp., producing a small inhibition zone (≈12 mm) only at 100% concentration, while lower concentrations were ineffective. This indicates a relatively low content of active antibacterial compounds or limited diffusion at reduced concentrations.

Similar to the results obtained with E. coli, Marrubium vulgare did not exhibit any antibacterial activity against Staphylococcus sp. under the tested conditions, suggesting that its ethanolic extract lacks effective antibacterial constituents or requires alternative extraction solvents or methods to enhance its activity.

The antibiotic controls again produced significantly larger inhibition zones, with penicillin showing the highest activity against Staphylococcus sp., followed by tetracycline, gentamicin, and amikacin. These results are in agreement with the known high sensitivity of Gram-positive bacteria to β-lactam antibiotics.

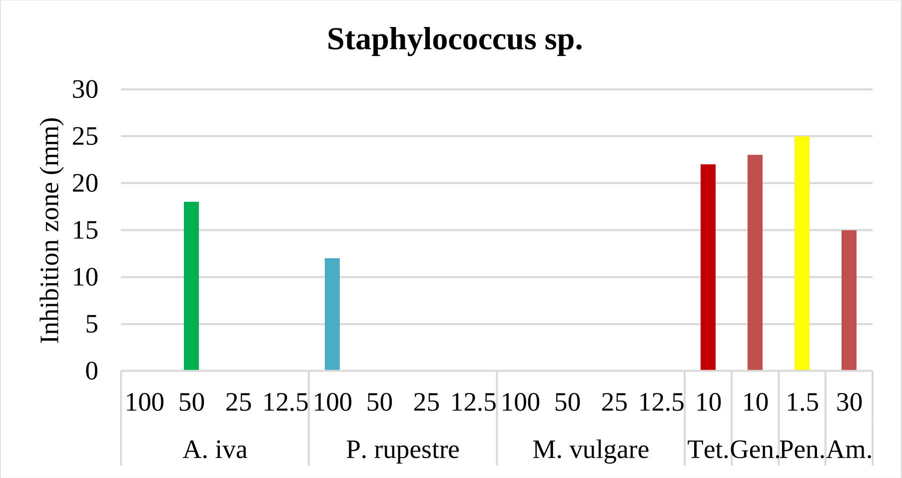

Fig. 4. Antibacterial activity of ethanolic extracts of Ajuga iva, Phagnalon rupestre, and Marrubium vulgare at different concentrations against Staphylococcus sp.

Comparative evaluation of plant extracts and antibiotics

Overall, the results demonstrate that the antibacterial activity of the tested plant extracts was considerably lower than that of standard antibiotics. Nevertheless, Ajuga iva showed promising antibacterial potential, particularly against Staphylococcus sp., indicating that this plant may serve as a potential source of natural antimicrobial compounds.

The observed differences in antibacterial activity among the tested plants may be attributed to variations in their phytochemical composition, such as phenolic compounds, flavonoids, terpenoids, and essential oils, which are known to possess antimicrobial properties. Additionally, the variation in activity across concentrations highlights the importance of optimizing extract concentration and extraction conditions.

The findings of this study confirm that Gram-positive bacteria are more susceptible to plant-derived extracts than Gram-negative bacteria. Among the tested plants, Ajuga iva exhibited the most notable antibacterial activity, while Phagnalon rupestre showed limited effects and Marrubium vulgare was inactive under the experimental conditions. Although the plant extracts were less effective than conventional antibiotics, the results support further investigation of Ajuga iva as a potential natural antibacterial agent, particularly through purification and identification of its active constituents.

For Ajuga iva, our results at 50% concentration showed inhibition zones of 13 mm for E. coli and 18 mm for Staphylococcus sp. These findings align with Makni et al. (2013) [3], who reported that A. iva methanol extracts were effective against S. aureus (20 mm) and E. coli (27 mm), though our results suggest that higher crude concentrations may contain inhibitory compounds that mask activity or require dilution to be effective. In contrast, our findings for Marrubium vulgare showed total resistance across all tested concentrations, which differs from the work of Ashkorfo et al. (2024) [12] and Soltani & Meddah (2021) [24], who reported inhibition zones ranging from 13 to 19 mm for E. coli and S. aureus. Similarly, Phagnalon rupestre only exhibited activity against Staphylococcus sp. at 100% (12 mm), remaining inactive against E. coli. This is consistent with Chikhi et al. (2019) [29], who noted that Phagnalon species often show much higher efficacy against Gram-positive bacteria like S. aureus than Gram-negative E. coli due to the protective outer membrane of the latter.

Conclusion

This study provides a comprehensive evaluation of the mineral profiles and antimicrobial potential of Phagnalon rupestre, Marrubium vulgare, and Ajuga iva. The elemental analysis revealed that all three species are significant sources of essential macro- and microelements, most notably calcium, which reached peak concentrations in M. vulgare (15,992 mg/L). Furthermore, the high iron content in M. vulgare and zinc in A. iva suggest these plants may serve as potent natural supplements for addressing micronutrient deficiencies. The detection of copper exclusively in P. rupestre (147 mg/L) further highlights the distinct, species-specific mineral storage capacities of these medicinal plants.

Regarding biological activity, A. iva demonstrated the most robust antimicrobial profile, exhibiting moderate inhibition of Escherichia coli and Staphylococcus spp. In contrast, P. rupestre showed selective activity against Staphylococcus spp., while M. vulgare lacked detectable antibacterial effects under the tested conditions. Although these natural extracts exhibited lower potency compared to conventional antibiotics such as tetracycline and gentamicin, the results offer scientific validation for their traditional ethnomedicinal applications.

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دراسة المعادن الأساسية والتأثيرات المضادة للميكروبات لبعض الأعشاب الطبية المختارة

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الخلاصة:

تم تحليل ثلاث نباتات طبية تُستخدم غالبًا في العلاجات التقليدية، وهي Phagnalon rupestre :، وMarrubium vulgare، وAjuga iva، بهدف تقييم محتواها المعدني ونشاطها المضاد للبكتيريا. تم جمع المواد النباتية من منطقة مسلاتة، ليبيا، ثم تم تنظيفها وتجفيفها هوائيًا لمدة 25 يومًا، قبل أن تُطحن وتُنخل وتُخزن في زجاجات محكمة الإغلاق. تم استخلاص 20 غرامًا من مسحوق كل نبات باستخدام 200 مل من الإيثانول في درجة حرارة الغرفة لمدة 72 ساعة، ثم تم ترشيح المستخلصات وتركيزها وتجفيفها عند درجة حرارة 40°C. أُجري التحليل المعدني باستخدام تقنية قياس طيف الامتصاص الذري و تقنية الانبعاث الذري اللهبي، وذلك بعد الهضم الرطب باستخدام حمض النيتريك المركز.(HNO_3) . ركز التحليل على ثلاثة عناصر كبرى (الصوديوم، والبوتاسيوم، والكالسيوم) وثلاثة عناصر صغرى (الحديد، والنحاس، والزنك). أظهرت النتائج أن نبات Ajuga iva احتوى على أعلى تركيز من الزنك (168.44 ملغ/ل)، في حين احتوى Marrubium vulgare على أعلى تركيز من الصوديوم (850 ملغ/ل) والحديد (4375 ملغ/ل). سجلت الأنواع الثلاثة مستويات مرتفعة من الكالسيوم، تراوحت بين 13,479 و15,992 ملغ/ل. كما أن النحاس لم يوجد إلا في Phagnalon rupestre بتركيز بلغ 147 ملغ/ل. أظهرت اختبارات النشاط المضاد للبكتيريا فعالية انتقائية ضد أنواع Staphylococcus وEscherichia coli. حيث أظهر Phagnalon rupestre تثبيطًا محدودًا ضد Staphylococcus فقط عند أعلى تركيز، بينما أظهر Ajuga iva تثبيطًا ملحوظًا ضد كل من E. coli وStaphylococcus عند تراكيز تتراوح بين 12.5% و50%. أما Marrubium vulgare، فلم يظهر أي نشاط مضاد للبكتيريا. كما هو متوقع، أظهرت المضادات الحيوية القياسية مثل الأميكاسين، الجنتاميسين، البنسلين، والتتراسيكلين مناطق تثبيط أكبر.

الكلمات المفتاحية: النباتات الطبية، مضاد للبكتيريا، محتوى المعادن، Marrubium، Phagnalon، Ajuga