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Phytochemical Analysis and antimicrobial activity of Five medicinal plants from Libya

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Abstract

The phytochemical profile and antimicrobial effectiveness of five native Libyan medicinal plants—Ephedra foeminea, Plantago uniflora, Ammi visnaga, Angelica polymorpha var. sinensis, and Tirmania species—are investigated in this study. The Soxhlet apparatus was used to extract plant components both aqueously and ethanolically. Numerous bioactive substances, with varying compositions among extract types, were found through qualitative phytochemical screening. These included Flavonoids, Alkaloids, Carbohydrates, Saponins, Cardiac, Glycosides, Coumarins, Tannins, Phenols, Proteins , Terpenoids and Resins. The extracts had mild acidity, with pH values ranging from 5.39 to 6.20. Staphylococcus aureus, Escherichia coli, Acinetobacter species and Staph aureus were among the Gram-positive and Gram-negative bacteria against which antibacterial activity was evaluated using the disk diffusion method. Significant inhibitory effects were shown by both ethanolic and aqueous extracts, especially against Streptococcus and E. coli, indicating potential for therapeutic uses.In addition to demonstrating the usefulness of Libyan flora as a source of innovative antibacterial agents, the diversity and richness of phytochemicals found in these species support their historic usage in ethnomedicine and highlight their pharmaceutical potential.

1.Introduction

Herbal medicine traces back to prehistoric times and remains one of the oldest therapeutic modalities (Sharifi‐Rad *et al.*, 2022). As infectious diseases continue to rise, the need for affordable, safe, and effective alternatives to synthetic antibiotics grows ever more critical. While conventional antibiotics are often effective, they can induce side effects and contribute to antibiotic resistance. In contrast, natural products, particularly those rich in phytochemicals such as flavonoids, phenolic acids, and β‐glucans, provide high biocompatibility and low toxicity, making them valuable therapeutic candidates (Sharifi‐Rad *et al.*, 2022).The

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phytochemical profiles and medicinal potentials of many Libyan endemic plants remain underexplored. Investigating species like Anagallis arvensis, commonly known as red pimpernel, is crucial. Its extracts contain phenolic acids such as salicylic acid, cinnamic acid, caffeic acid, and flavonoids (Sharifi‐Rad et al., 2020), which have demonstrated inhibitory effects on plant germination and possess various biological activities. Traditionally, A. arvensis has been used to treat ailments including gout, epilepsy, rheumatism, and renal dysfunction (Sharifi‐Rad *et al*., 2020; Sharifi‐Rad et al., 2015). Moreover, β-glucans isolated from the pollen of Phoenix dactylifera (date palm) have shown significant anticancer potential through (1→3)-β-D-glucan linkages (Eshward & Kennedy, 2005). These compounds, along with date polyphenols, can modulate critical signal transduction pathways such as NF‐κB, suppressing inflammation and oxidative stress via antioxidant action (Jaouhari et al., 2024; Khalifa & Aldhafiri, 2023). Date leaves and fruits are rich sources of antioxidants and anti-inflammatory agents that may regulate immune responses. Aqueous extracts of P. dactylifera fruits have been shown to enhance cell-mediated immunity and exhibit therapeutic effects in traditional medicine .The palm's historic use in treating infections, fever, neurological disorders, and paralysis further supports its ethnopharmacological relevance (El Abed et al., 2018).This study aims to perform a comprehensive phytochemical screen of selected Libyan medicinal plants (Phoenix dactylifera, Anagallis arvensis), targeting key bioactive classes: phenolic acids, flavonoids, alkaloids, cardiac glycosides, terpenoids, saponins, tannins, carbohydrates, and coumarins. Detecting these compounds will validate traditional uses and guide future pharmaceutical investigations.

2. Material and Methods

2.1. Plants Sample Collection

Leaves of Ephedra Foeminea, Plantago uniflora, Ammi visnaga, Angelica polymorpha var. sinensi, and Tirmania were procured from the Zliten region in Libya. The plant samples were authenticated by the Biology Department, College of Science, Alasmariya University, and voucher specimens were deposited in the university herbarium

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2.1.1 pH measurement

Approximately 3 g of the dry extracts were dissolved in 30 mL of distilled water in a 100 mL flask. The pH was measured using a HANNA Instruments pH meter at 25 °C. The results are presented in Table 1. Standard protocols for pH analysis of plant extracts advise using a calibrated glass electrode and filtered aqueous solutions to ensure accurate measurement at 25 °C (Chen *et al*., 2022).

2.2. Extraction of Plant Material

Twenty grams of finely powdered plant parts were extracted using a Soxhlet apparatus with 500 mL of either aqueous or ethanolic solvent separately. The extraction was performed at a 40% (w/v) concentration (20 g leaves powder in 500 mL solvent). Each extract was subsequently filtered and stored at 4 °C in a refrigerator until further use (Krishnaiah, 2009).

Soxhlet extraction remains a gold-standard for recovering both polar and non‑polar secondary metabolites due to its efficiency and reproducibility (Bitwell, 2023).

2.3. Qualitative Phytochemical Analysis

Aqueous and ethanolic extracts were qualitatively analyzed for phytochemical constituents following standard procedures described by Krishnaiah (2009). These include classical colorimetric and precipitation-based assays for major phytochemical groups, widely validated in modern phytochemical research (Maheshwaran *et al*., 2024).

1. **Flavonoids**: Presence confirmed by boiling 20 mL extract with ammonia + H_2SO_4 (yellow color) or NaOH → HCl (disappearing yellow). Flavonoid detection via ammonia/H2SO4 and sodium hydroxide reagents is recognized as reliable in preliminary screening (Maheshwaran *et al*., 2024).
2. **Alkaloids:** Detected using Dragendorff's, Mayer's, and Wagner's reagents, producing characteristic precipitates. These classical tests are still widely accepted for qualitative alkaloid identification (Maheshwaran *et al*., 2024; Iqbal, 2015).
3. **Carbohydrates**: Fehling's Test (violet ring) and Molisch's test confirm reducing sugars/carbohydrates. Standard protocols confirm these as robust qualitative indicators (Maheshwaran *et al*., 2024).
4. **Saponins**: For Olive's oil test, 5ml of distilled water was added to 20 ml of extract, followed by a few drops of olive oil and shaking for 3 minutes. The appearance of froth and stable foam indicated saponins. Mercuric Chloride ($HgCl_2$) test involved adding 3ml of Mercuric Chloride solution to 5 ml of extract; a white precipitate confirmed saponins (Maheshwaran *et al*., 2024).
5. **Cardiac Glycosides**: The Keller-Killani Test was performed by treating 20 ml of each extract (separately) with 8 ml of glacial acetic acid containing one drop of ferric chloride solution, followed by shaking. This was then underlaid with 1 ml of concentrated Sulphuric acid. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring, and a greenish ring might gradually form in the acetic acid layer (Iqbal, 2015)
6. **Coumarins**: 5ml of extract was placed in a test tube, covered with wet filter paper saturated with sodium hydroxide solution (NaOH), and heated in a water bath for a few minutes. Exposure to ultraviolet light revealed a greenish-yellow color, indicating the presence of coumarins (Maheshwaran *et al*., 2024)
7. **Tannins**: For Ferric Chloride ($FeCl_3$) test, 10 ml of extract was boiled, and a few drops of Ferric Chloride solution were added to the filtrate. A bluish-green color indicated tannins. Lead Acetate $Pb(C_2H_3O_2)_2$ test involved adding 10 ml of Lead acetate solution to 3ml of the extract and shaking for 2 minutes; a gel precipitate indicated tannins (Iqbal, 2015).
8. **Phenols**: 10ml of extract was treated with 4-5 drops of ferric chloride solution. The formation of a bluish-black color indicated the presence of phenols(Maheshwaran *et al*., 2024)
9. **Proteins** :Biuret's Reagent: A few drops of biuret's reagent were added to 10ml of the extract; the appearance of a pink color in the ethanolic layer indicated proteins. (Iqbal, 2015)

10. **Terpenoids**: Salkowski's test involved adding 5ml of chloroform to 10ml of extract, followed by 2ml of concentrated Sulphuric acid (H_2SO_4) with care, and heating for 2 minutes. A reddish-brown coloration at the interface indicated terpenoids (Iqbal, 2015)
11. **Resins**: 10ml of ethyl alcohol was added to 20ml of the extract, heated, and filtered. Subsequently, 10ml of diluted hydrochloric acid (HCl) was added; turbidity indicated the presence of resin(Maheshwaran *et al*., 2024)

2.4. Determination of Antibacterial

Bacterial Strains and Culturing: The concentrated plant extracts were transferred from the Department of Chemistry, Faculty of Science, Alasmareia University, Zliten, Libya, to the Department of Microbiology Laboratory at Zliten Teaching Hospital, Zliten, Libya. Four pathogenic bacterial strains were utilized in this study: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumonia. These strains were cultured at 37°C(Mohamed *et al*., 2020).

2.4.1 Determination of Minimum Inhibitory Concentration (MIC): Fifty microliters of bacterial culture (OD600 = 1.0) were inoculated into each LB media tube containing 10 to 150 mg/ml of the sample. Two negative controls were included: one with LB broth only and another with LB broth and extract (100 mg/ml). A positive control consisted of LB broth and the test organism. After 24 hours of incubation at 37°C, the absorbance of the suspension was measured using a spectrophotometer at a wavelength (lambda) = 600 nm. The concentration of the test sample at which bacterial culture growth was inhibited was considered the MIC(Kadeřábková *et al*., 2024).

2.4.2 Disc-Diffusion Assay:

The disc-diffusion assay was conducted to assess activity against bacterial strains. Lysogenic Broth (LB) agar plates were inoculated with bacterial culture (250 mul bacterial culture with OD600 of approximately 1 per 100 ml LB media). Four discs, approximately 6mm apart, were used. Consistent broth inoculums were swabbed onto sterile LB agar plates using sterile cotton swabs. Sterile paper discs (6 mm) were saturated with aqueous and ethanolic leaf extracts separately (pre-dissolved in 10% dimethyl sulphoxide (DMSO)) to achieve ultimate extract concentrations per disc. Two sterile paper discs (6 mm) loaded with solvents (aqueous and ethanolic) were used as growth controls. The discs were aseptically and individually placed onto the inoculated LB agar plates. The plates were incubated at 37°C for 16-18 hours, and the resulting inhibition zones were measured and compared with those of antibiotics (Nitrofurantoin). The test was performed in triplicates(Balouiri *et al*., 2015).

Agar-Well Diffusion Assay: The agar-well diffusion assay was also performed to determine activity against bacterial strains. LB agar plates were inoculated with bacterial culture (250 mul bacterial culture with OD600 of approximately 1 per 100 ml LB media). Four wells, approximately 6mm in diameter, were created using a 6 mm cork borer. The first and second wells received 50 mul of the diluted aqueous and ethanolic extracts (100 mg/ml) separately. The third and fourth wells were loaded with controls: 50 mul of solvents (water and ethanolic extract) as negative controls and 50 mul of Nitrofurantoin (40 mg/ml) as a positive control, respectively. The plate was then incubated at 37°C overnight, and the diameter of the zone of inhibition was measured in millimeters. This procedure was followed for all extracts, and the experiment was replicated thrice(Hulankova, 2024).

3. Results and Discussion

Numerous bioactive secondary compounds—such as flavonoids, alkaloids, saponins, cardiac glycosides, phenols, tannins, proteins, and terpenoids—were identified in the crude extracts (aqueous and ethanolic) of the selected plant species. These compounds are closely associated with the extracts' pronounced biological activities, notably their antibacterial efficacy. These findings align with the comprehensive review by Jaiswal et al. (2024), which demonstrated that Leopoldia comosa bulbs contain a rich diversity of phytochemicals—particularly flavonoids and homoisoflavones—and exhibit potent antioxidant, antibacterial, anti-inflammatory, anticancer, and immunostimulatory effects (Jaiswal *et al*., 2024)

Table 1: phytochemical screening of Qualitative analysis

Plant's Name	Ephedra Foeminea		Plantago uniflora		Ammi visnaga		Angelica polymorpha var. sinensi		Tirmania	
Chemical Constituents	EtOH Extra.	Aqua. Extra	EtOH Extra.	Aqua. Extra	EtOH Extra.	Aqua. Extra	EtOH Extra.	Aqua. Extra	EtOH Extra.	Aqua. Extra
Flavonoids	+++	++	+	++	++	+++	+++	+++	++	++
Alkaloids	+++	++	+	++	++	+++	++	++	+	++
Carbohydrates	+++	+	+++	++	+++	+	++	+	++	++
Saponin	+++	+	++	++	+	++	++	++	++	+
Cardiac Glycosides	++	+	++	++	+++	+++	+++	+++	++	+
Coumarins	+++	+	++	+++	+++	++	+++	++	++	+
Tannins & Phenols	++	-	+	++	++	+++	+++	+++	++	+
Proteins	+++	+	+	++	+++	+	++	-	++	+
Terpenoids	+++	+	++	-	++	-	+++	+	++	-
Resins	+++	+	-	++	++	+++	+	+	+	++
pH	5.39		6.20		6.12		5.73		6.19	

Legend: +++ denotes: presence in rich; ++ denotes: moderate; + denotes presence; - denotes: absence

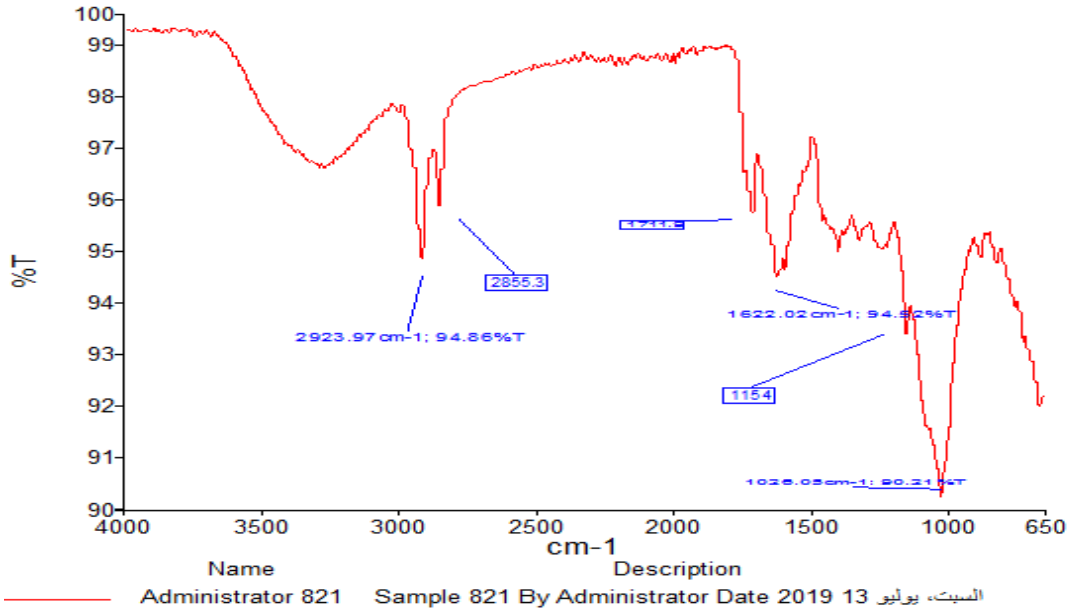

Fig 1: Results of analysis of Infrared (IR) Spectra for Ammi visnaga

Ammi visnaga. IR spectroscopy is a method used to identify active organic compounds and assess their purity by analyzing beam sites and peaks. The infrared spectra showed absorption characteristics of nicotine, where the vibration frequencies of key active groups in the compound were identical to those of standard plants. A strong beam appeared at 2921 cm^{-1}, indicating an implicit hydrogen bond between hydroxyl and carbonyl groups. A weaker beam was observed between 2917 - 3400 cm^{-1}. Peaks between 2102 – 2921 cm^{-1} correspond to aromatic C-H bonds. An additional strong and sharp carbon group C=H beam appeared between 1450 – 1017 cm^{-1}. Another beam between 1380 -1447 cm^{-1} exhibited medium sharpness, indicative of C=C aromatic bonds. A strong and sharp C-N beam emerged between 604 and 1017 cm^{-1}. Furthermore, a strong and sharp beam at 1719 cm^{-1} represented a bending frequency for a C=N group. Several characteristic peaks for different chemical functions of nicotine were observed on this spectrum. Around 3400 cm^{-1}, a large peak corresponds to the water molecule (consistent with a liquid film). C-H stretching was observable between 2921 and 2970 cm^{-1}. A prominent peak at 1677 cm^{-1} indicates the expansion of the aromatic C=N bond. Similarly, another peak at 1691 cm^{-1} represents the extension of aromatic C=C bonds. The peaks at 717 cm^{-1} and 904 cm^{-1} correspond to out-of-plane bending or swinging of the C-H bond of mono-substituted Pyridine rings. The findings demonstrated that, in terms of antibacterial activity, the ethanolic extracts performed better than the aqueous extracts. Leopoldia comosa's ethanolic extract, for instance, demonstrated the largest inhibition zone of 18 mm against Pseudomonas aeruginosa and E. coli. A prior study that showed that alcoholic extracts could extract active compounds with antibacterial activity and was published in The Pharma Innovation Journal in 2023 supports these findings [Opwoko *et al*., 2023]. In a similar vein, Anagallis arvensis extracts demonstrated promising outcomes, with an inhibitory zone of 15 mm in the ethanolic extract and just 7 mm in the aqueous extracts. These findings are in line with a study on the same plant that demonstrated that its alcoholic extracts successfully

stopped Staphylococcus aureus from growing, with an inhibition diameter of 9–11 mm. Because ethanolic extracts of palm pollen include significant levels of phenols and antioxidant chemicals, they have been shown to be effective against both Gram-positive and Gram-negative bacteria, including Pseudomonas aeruginosa. These extracts' minimal inhibitory concentrations (MICs) ranged from 435 to 495 µg/ml. When compared to other studies, such as those showing the efficacy of various medicinal plants with an average MIC range of 200 to 1200 µg/mL, these values are considered acceptable. The efficiency of crude plant extracts generally reflects the abundance of bioactive compounds, lending support to their traditional use as natural antibiotics in folk medicine. These findings highlight the promise of plant-derived extracts as complementary or alternative sources to synthetic antibiotics, especially given the growing challenges of antibiotic resistance (Jahan *et al*., 2023).

Table 2: Diameters inhibiting antibiotic evidence on the Types of Bacterial

Type of Bacteria Plant		***Staphylococcus aureus***	**Escherichia Coli**	***Acinetobacter* species**	**Staph aureus**
Ephedra Foeminea	**EtOH Extra**	13	12	12	10
	Aqua. Extra	-	-	-	-
Plantago uniflora	**EtOH Extra**	15	13	10	11
	Aqua. Extra	-	-	-	-
Ammi visnaga	**EtOH Extra**	16	13	11	11
	Aqua. Extra	-	-	-	-
Angelica polymorpha var. sinensi	**EtOH Extra**	19	-	-	-
	Aqua. Extra	-	-	-	-
Terfezia and Tirmania	**EtOH Extra**	17	10	-	-
	Aqua. Extra	-	-		-
Legend: (mm) = Millimetre					

The different levels of methicillin-resistant Staphylococcus aureus (MRSA) susceptibility to the plant extracts under study demonstrate how intricate and unique the phytochemical makeup of these extracts is. Higher amounts of active substances like alkaloids and saponins, which have demonstrated strong antibacterial qualities in earlier research, are thought to be responsible for these variations in bioactivity. The suppression of bacterial enzymes by oxidizing chemicals, which are generators of unstable free radicals (ROS), is one theory as to how these substances work. Critical bacterial proteins are disrupted and cell membranes are damaged as a result of these radicals' interactions with proteins and cellular structures (Ahmed *et al*., 2023; Batiha *et al*., 2020). In addition to their direct interaction with the bacterial cell wall, some of these chemicals have also been demonstrated to interfere with soluble extracellular proteins, causing membrane lysis and the termination of vital bacterial life processes (Nasir *et al*., 2022). The plant Leopoldia comosa is unique from an

ethnobotanical standpoint because of its long history of use in the southern Italian region of Basilicata, where it was used to treat toothaches, headaches, and infections of the skin and soft tissues. This supports the plant's current therapeutic potential as an antibacterial (Rivara *et al*., 2021). The bulbs of this plant, which is native to the Mediterranean region, have long been used as food. According to recent studies, it includes active alkaloids and polyphenolic chemicals that have antioxidant and antibacterial properties (Zengin *et al*., 2024). These findings are in line with the study's findings, which demonstrated that the plant's ethanolic extracts had exceptional efficacy against Staphylococcus aureus. This suggests that the plant may one day be developed into an antibacterial agent, particularly in light of the spread of antibiotic resistance.

4. Conclusion

This study underscores the critical need for further biochemical investigations, including the isolation and identification of plant components, and their potential applications in enhancing scientific field reviews. Such efforts are crucial for evaluating the efficacy of traditional herbal medicine in treating conditions like diabetes and other diseases. Furthermore, the current research suggests that Leopoldia comosa, Palm-pollen, and Anagallis arvensis are promising sources of natural antioxidants and thus hold significant therapeutic potential.

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التحليل الكيميائي النوعي والنشاط المضاد للميكروبات لخمسة انواع من النباتات الطبية في ليبيا

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استلمت الورقة بتاريخ 2025/08/10، وقبلت بتاريخ 2025/08/19، ونشرت بتاريخ 2025/08/23

الكلمات المفتاحية:
الفحص الكيميائي النباتي ، النباتات الطبية ، النشاط المضاد للبكتيريا ، منطقة التثبيط ، النباتات الليبية.

الملخص

المظهر الكيميائي النباتي (المواد الفعالة) والفعالية المضادة للميكروبات لخمسة نباتات طبية ليبية محلية, وهي (إيفيدرا فومينا ، بلانتاجو يونيفلورا ، أمي فيسناغا ، أنجليكا بوليمورفا فار .سينينسيس ، وأنواع التيرماني) تم التحقيق منها في هذه الدراسة . حيت تم استخدام جهاز سوكسليت لاستخلاص المكونات النباتية بواسطة الماء و إلايثانول و تم العثور على العديد من المواد النشطة بيولوجيا مع تركيبات مختلفة بين أنواع المستخلصات من خلال الفحص الكيميائي النباتي النوعي .وشملت العفص ، الفلافونويد ، الفينولات ، الصابونين ، قلويدات ، الكربوهيدرات ، جليكوسيدات ، الكومارين ، والراتنجات والبروتينات والتربينات. وكانت قيم الأس الهيدروجيني للمستخلصات تتراوح من 5.39إلى 6.20 وكانت المكورات العنقودية الذهبية والإشريكية القولونية وأنواع الأسينتوباكتر والمكورات العنقودية الذهبية من بين البكتيريا موجبة الجرام وسالبة الجرام التي تم تقييم النشاط المضاد للبكتيريا ضدها باستخدام طريقة انتشار القرص .تم عرض تأثيرات مثبطة كبيرة من قبل كل من المستخلصات الإيثانولية والمائية و خاصة ضد المكورات العقدية و القولونية ، مما يدل على الإمكانات العلاجية بالإضافة إلى إظهار فائدة النباتات الليبية كمصدر للعوامل المضادة للبكتيريا المبتكرة ، تنوع وثراء المواد الكيميائية النباتية الموجودة في هذه الأنواع يدعم استخدامها التاريخي في الطب الشعبي ويسلط الضوء على إمكاناتها الصيدلانية .