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Phytochemical Screening and antioxidant activity antibacterial activity for *Ephedra altissima* plant growing in city of Alkums libya

Ismail M. Awheda[1]* , Fathi A. Smida [2] , Aisha Abdullah Salem Fattoul [3]
[1.2.3] Chemistry Department, Faculty Of Science, Al-Mergib University, Alkhoms, Libya.
imawaheda@elmergib.edu.ly

دراسة المحتوى الكيميائي النباتي وبعض محتويات مضادات الأكسدة والنشاط البيولوجي لنبات العلندة بمنطقة قوقاس- الخمس- ليبيا

إسماعيل مختار أوحيدة *[1]، فتحي أحمد صميدة [2]، عائشة عبدالله سالم فتول [3]
[1.2.3] قسم الكيمياء ، كلية العلوم ، جامعة المرقب ، الخمس ، ليبيا

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Abstract:
This study aimed to estimate the natural products in the plant *Ephedra altissima* by qualitatively assessing its chemical compound content through tests for chemical groups in both aqueous and ethyl acetate extracts. The yield of extraction using water was 12.881%, which was higher compared to the yield using ethyl acetate at 3.73%. The chemical analysis of the aerial parts of the studied plants (*Ephedra altissima*) revealed the presence of compounds such as alkaloids, carbohydrates, saponins, phytosterols, tannins, flavonoids, proteins, and terpenoids. The chemical analysis of the aqueous extract of *Ephedra altissima* showed the presence of alkaloids, carbohydrates, saponins, phytosterols, flavonoids, and terpenoids, while the ethyl acetate extract contained alkaloids, carbohydrates, phytosterols, tannins, flavonoids, proteins, and terpenoids.
It was noted that the aqueous extract of *Ephedra altissima* did not contain proteins or tannins, while saponins were not detected in the ethyl acetate extract.
Quantitative and qualitative estimations of chemical compounds were conducted using Gas Chromatography-Mass Spectrometry (GC-MS), which revealed the presence of several compounds in significant quantities in the studied plant. The results indicated that the aerial parts of *Ephedra altissima* contained 26 chemical substances, with the most abundant compounds being: 28.76% Hexadecanoic acid, methyl ester and 34.74% Hexadecatrienoic acid, methyl ester. The highest abundant compound in the aerial parts of the caper plant was 9-Octadecenoic acid (Z)-, methyl ester.
Furthermore, antioxidant activity was studied using the DPPH test to estimate the antioxidant effectiveness, with the results showing that the extract of *Ephedra altissima* had a low IC50 value, indicating increased antioxidant activity of the sample.

Thus, it can be concluded that the aerial parts of *Ephedra altissima* exhibit antioxidant activity with a value of approximately 13.49 µg/mL. When comparing these results with ascorbic acid (8.041), which is used as an antioxidant, it can be said that the extracts showed lower antioxidant activity than the reference compound.
Finally, the antibacterial effects of the plant extracts were studied by testing their antibacterial effectiveness against two pathogenic bacterial strains: *Staphylococcus aureus* and *Escherichia coli*. The results showed that the aqueous extract of *Ephedra altissima* demonstrated inhibitory activity against the gram-negative *Escherichia coli*, with a larger inhibition zone of 10 mm compared to the ethyl acetate extract, which had an inhibition zone of 6 mm. Conversely, the inhibition activity of the aqueous extract against gram-positive *Staphylococcus aureus* showed lower inhibitory activity with a zone of 6 mm compared to the ethyl acetate extract, which had an inhibition zone of 12 mm.

Keywords: Ephedra altissima, Escherichia coli , Staphylococcus aureus, alkaloids, antioxidant, flavonoids.

الملخص:
خلصت هذه الدراسة إلى تقدير المنتجات الطبيعية في نبتة العلندة، وذلك عن طريق التقدير النوعي لمحتواها من المركبات الكيميائية من خلال إجراء اختبارات الكشف عن المجاميع الكيميائية لكل من المستخلص المائي ومستخلص خلات الإيثيل حيث كانت مردودية الاستخلاص باستخدام الماء هي (12.881%) التي كانت أعلى مقارنة بمردودية الاستخلاص باستخدام خلات الإيثيل حيث كانت (3.73%). كذلك بين المسح الكيميائي لمستخلص الأجزاء الهوائية لنباتات الدراسة (العلندة) احتواءه على مركبات (القلويدات والكربوهيدرات والصابونين والفيتوسترول والتنينات والفلافونويدات والبروتينات والتربينات). حيث أظهر المسح الكيميائي لمستخلص الماء للعلندة وجود القلويدات والكربوهيدرات والصابونين والفيتوسترول والفلافونويدات والتربينات، بينما احتوى المستخلص خلات الإيثيل والقلويدات والكربوهيدرات والفيتوسترول والتنينات والفلافونويدات والبروتينات والتربينات.
ولوحظ أن المستخلص المائي للعلندة لا يحتوي على البروتينات والتنينات، بينما لم يتم الكشف عن وجود الصابونين في مستخلص خلات الإيثيل للعلندة.
تم التقدير الكمي والكيفي للمركبات الكيميائية باستخدام تقنية كروماتوغرافيا الغازية المرتبطة بمطياف الكتلة (GC-MS)الذي أظهرت نتائجه وجود بعض المركبات بكميات معتبرة في النبات قيد الدراسة. حيث أظهرت نتائج المسح لمستخلص الأجزاء الهوائية لنبات العلندة وجود عدد (26) من المواد الكيميائية، وكان من أعلى المركبات وفرة بها هي Hexadecanoicacid, methyl ester (28.76%) Hexadecatrienoic acid, methyl ester (34.74%)، بينما أعلى المركبات وفرة في مستخلص الأجزاء الهوائية لنبات القبار كانت 9-OCTADECENOIC ACID (Z)-, METHYL ESTER.
كذلك تمت دراسة النشاط المضاد للأكسدة عن طريق اختبارDPPH ، وذلك لغرض تقدير الفعالية المضادة للأكسدة. حيث سجلت النتائج لمستخلص نبات العلندة قيمة IC50 منخفضة، مما يعني زيادة نشاط مضادات الأكسدة للعينة.
لذا يمكن القول إن مستخلص الأجزاء الهوائية لنبات العلندة له نشاط مضاد للأكسدة بقيمة 13.49 ميكروغرام/مل تقريباً. عند مقارنة النتائج التي تم الحصول عليها مع حمض الأسكوربيك (8.041) الذي يستخدم كمضاد للأكسدة، يمكن القول إن المستخلصات أعطت نشاطًا مضادًا للأكسدة أقل من المركب المرجعي.

الكلمات الدالة: العلندة، الفعالية، المضادة للأكسدة، الفعالية المضادة للبكتيريا.

Introduction

"Medicinal plants have been an integral part of traditional and modern healthcare systems. Throughout history, ancient civilizations relied on herbs and plants for treating diseases and promoting health. For example, the Ebers Papyrus, dating back to ancient Egypt, is one of the earliest records documenting the use of medicinal plants (1). Traditional Chinese medicine and Indian Ayurveda also incorporated numerous herbs, many of which are still studied and used today.

Medicinal plants contain a wide range of natural chemical compounds, such as alkaloids, flavonoids, and essential oils, which confer anti-inflammatory, antioxidant, and antimicrobial properties (2). These compounds form the scientific basis for many modern drugs developed from plant sources, such as aspirin derived from willow bark and quinine used to treat malaria (3).

With scientific advancements, the study of medicinal plants has become a focal point in pharmaceutical and biochemical research. These studies aim to identify bioactive compounds and analyze their effects on human health, contributing to the development of new and effective medications. However, improper or unsupervised use of medicinal plants can lead to adverse side effects, emphasizing the importance of safe and sustainable use (4).

Medicinal plants are not only a source of therapeutic value but also represent a connection between humans and nature, demonstrating their ability to provide sustainable and effective health solutions. This makes them an essential part of both human health and environmental conservation."

A Marvel of Adaptation and Medicinal Value

Ephedra altissima, commonly known as the scarlet ephedra, is a fascinating plant belonging to the genus *Ephedra* and the family *Ephedraceae*. This perennial shrub is renowned for its striking crimson flowers and slender, jointed stems, which allow it to stand out in the harsh environments where it thrives. Found primarily in arid and semi-arid regions of North Africa and parts of the Mediterranean, *Ephedra altissima* has adapted remarkably to survive in extreme conditions, making it a subject of great ecological, medicinal, and scientific interest.

From an ecological perspective, *Ephedra altissima* plays a critical role in stabilizing fragile desert ecosystems. Its deep root system anchors it firmly in rocky or sandy soils, preventing erosion and promoting soil health in regions where vegetation is sparse (5). The plant’s drought-resistant features, such as its reduced leaf surface and efficient water storage mechanisms, enable it to minimize water loss, a key adaptation to arid climates (6). Moreover, *Ephedra altissima* supports local biodiversity by providing shelter and food for various insects and small animals, further highlighting its ecological significance.

Medicinally, *Ephedra altissima* has been valued for centuries. Like other members of the *Ephedra* genus, it contains alkaloids such as ephedrine and pseudoephedrine, compounds known for their bronchodilator properties (7). These alkaloids have been used traditionally to treat respiratory ailments, including asthma, colds, and bronchitis. Modern pharmacological studies have confirmed the efficacy of these compounds, leading to their incorporation into numerous over-the-counter medications (8). However, the use of *Ephedra*-derived compounds requires careful regulation due to potential side effects, including increased heart rate and blood pressure.

In addition to its medicinal applications, *Ephedra altissima* holds cultural significance in traditional medicine systems. Communities in regions where the plant grows have used it not only for respiratory health but also as a general stimulant and diuretic. Its inclusion in ancient remedies underscores its enduring importance to human health and wellbeing (1).

Scientifically, *Ephedra altissima* serves as a valuable model for studying plant survival in extreme conditions. Researchers have investigated its photosynthetic efficiency under high

temperatures and limited water availability, gaining insights into its remarkable resilience (9). Such studies have broader implications, particularly for developing drought-resistant crops in the face of climate change. The plant's ability to thrive in marginal environments provides a blueprint for sustainable agriculture in arid and semi-arid regions.

Despite its numerous benefits, *Ephedra altissima* faces challenges, including habitat loss due to urbanization and overharvesting for medicinal purposes. Conservation efforts are essential to protect this species and its habitat, ensuring its ecological and medicinal contributions remain available for future generations (10).

In conclusion, *Ephedra altissima* is more than just a desert shrub. It is a resilient survivor, a healer, and a source of inspiration for scientists and conservationists alike. Its ecological role, medicinal value, and adaptability to harsh conditions make it an indispensable part of the natural and cultural heritage of the regions it inhabits. Continued research and conservation efforts will ensure that this remarkable plant continues to thrive and benefit humanity

Materials and methods

1- (GC-MS) analysis method

The chemical composition of the samples was performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30m x 0.25mm x 0.25µm film thickness). The column oven temperature was initially held at 50 C and then increased by 5°C /min. to 230°C then held for 2min. increased to the final temperature 290°C by 30°C /min. and held for 2min. The injector and MS transfer line temperatures were kept at 250, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using Auto sampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C, and then The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database (11).

2- Antioxidant Activity Test

Since DPPH is based on coloring and decolorizing at a certain wavelength, this method has been applied to determine the captivating activity of plant antioxidant compounds.

Evaluation of antioxidant activity by DPPH radical scavenging method, this method is also known as the BLOIS anti-free radical test, measures the capacity of extracts (antioxidants) to donate a hydrogen atom (12). The colorimetric reaction of the free radical DPPH indicates if the test is inhibiting the free radical.

The ability of sample extracts to scavenge free radicals were assessed using 1,1-diphenyl-2-picryl hydrazyl (DPPH). 0.1 of DPPH solution in ethanol was made3.0mlof various extracts in ethanol with different concentrations 3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/ml were mixed with 1.0ml with this solution.

The only extracts that dissolve in ethanol are employed here, and the dilution procedure was used to create the various concentrations of the extracts. After giving the mixture a good shake, it was left at room temperature for half an hour. The absorbance was then determined at 517nm using a UV-VIS Milton Roy spectrophotometer. The experiment was conducted in triplicate using ascorbic acid as the reference standard component (13). The IC50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. A lower absorbance reaction in the mixture indicates higher free radical activity. The percent of DPPH scavenging effect was calculated using following equation:

DPPH scavenging effect (%) or Inhibition Percentage = (A0 - A1 / A0) × 100, where A0 is the Absorbance of control reaction and A1 the Absorbance in presence of test or standard sample.

3- Anti-bacterial test

In this investigation, a variety of common human infectious bacterial strains were used to examine the efficacy of their category (14) as shown in table (1).

Table 1: Bacterial strains tested in the research

Type of bacterial strain	Symbol of isolation	Classification
Staphylococcus aureus	S.aureus	Gram Positive
Escherichia coli	E.coli	Gram Negative

The antibacterial activity of the plant extracts were assessed using the discs on agar medium method. Microorganisms Escherichia coli and Staphylococcus aureus were cultured on an agar medium plate in an incubator for a whole day after being isolated from a urine sample. The Gram strain was used for the identification.

All of the used to.ols were sterilised, and the area surrounding the Bunsen burner was carefully cleaned filter paper discs was then applied as following procedure (15):

Preparation of bacterial suspension; the bacterial fragment was taken with a cotton swab and put in a tube with 3ml of sterile physiological water to prevent the bacteria from being damaged. The solution was then shaken well over a gasoline burner. Next, the bacterial species were cultured in a petri box, where the bacteria were eventually distributed over the growth media surface using a cotton swab, and then it was labelled.

Preparing of the tablets; after cutting the Whatman filter papers into 6 mm-diameter discs, the discs were put in a glass test tube with 10 ml of distilled water. The glass test tube was then autoclaved for 20 minutes at 120 °C, the water was withdrawn, and the discs were moved to an incubator for drying. When you are ready to evaluate the extracts' impact on different bacterial strains, immerse these tablets in a variety of extracts until they are fully saturated.

Preparation of Extracts on Agar Plates, using sterile forceps, discs saturated with extracts were placed into Petri dishes. The discs were positioned evenly spaced from the edge of each dish. Subsequently, the Petri dishes were inverted and incubated for 24 hours at a temperature of 37 °C. Once removed from the incubator, the distance to the nearest bacterial growth was measured.

Results and Discussion

1- Sample preparation

The study plants were collected from the Umm Al-Rathm region in the Libyan municipality of Qawqas-Al-Khoms. The plant samples underwent a series of procedures including cleaning; room temperature drying, grinding, sieving as in **figure (1)**, and they were stored in darkened sealed bottles marked with the names of the plants and the collection date.

The powdered aerial parts of *Ephedra altissima* possess a coarse texture attributed to the plant's fibres, resulting in a gritty powder that has an astringent flavour and a pine scent.

Figure 1: The aerial parts of the study plant before and after grinding

2- Moisture percentage

The experimental investigation found that the percentage of moisture and volatile material lost through drying in the aerial parts of the *Ephedra altissima* plant was 30.4%, which indicating the volatile chemical contents of the plant.

According to the experimental results, the *Ephedra altissima plant's* aerial parts have percentage of ash 10%, which reflect the content of inorganic materials, or minerals, in the plant's aerial parts.

3- pH measurements

The measurement showed that the aerial parts of the *Ephedra altissima* plant have a pH of 4.66. The measurements indicate the acidic influence of the aerial parts of the plant.

4- Yield of extractions

The soaking method was applied, along with a shaking device set to 100 vibrations per minute at room temperature for 24 hours; the process is a calculation method in terms of the mass of the dry plant material used and the mass of the extracted dry plant material by water and ethyl acetate separately. Water is used due to the high polarity to enhance extracting the polar materials.

Recent advancements in phytochemical research emphasize ethyl acetate's important role as an intermediate-polarity solvent in plant extraction. Ethyl acetate is effective in isolating bioactive compounds, particularly flavonoids and phenolics, due to its polarity index of 4.4 and boiling point of 77.1°C. (16) In Phytochemical Analysis found that ethyl acetate-based extractions achieve higher recovery rates of medium-polarity compounds, reaching up to 95% efficiency.(17)

The yield of the extraction was determined for the study plant (*Ephedra altissima*), as displayed in **table (2)**.

Table 2: The percentage and the color of the extracts

Plant	Ethyl acetate extract		Aqueous extract		Volume of solvent (ml)	weight of Sample (g)
	Color	Productivity%	Color	Productivity%		
E. altissima	Green	3.73%	Reddish/brown	12.881%	100	10

The aerial parts of the studied plants were extracted by shaking for 24 hours at room temperature. The extraction technique was employed in two separate polar solvents: distilled water and ethyl acetate. According to the preceding table, the aqueous extract of the studied plant yielded 12.881%, while the ethyl acetate extract yielded 3.73%. The variation of the extraction yields depends on the polarity of the solvent used; because of that, water extracted more materials than ethyl acetate; moreover, the yields of the extraction reflect the high content of polar compounds found in the plant.

5- Phytochemical screening

Chemical detection tests were used to identify the chemical compounds found in the aerial parts of the studied plants. Specific reagents to each family of active compounds are used for the identifications, which are kind of reactions depending on the precipitate or colour change occurring. **Table (3)** displays the findings of the detection tests performed on *E. altissima* extracts.

Table 3: Phytochemical screening of *Ephedra altissima* extracts

Extract / Test		H_2O	EtAc
Alkaloids	Wagner	+	+
	Dragendroff	+	+
Carbohydrates	Molisch	+	+
	Benedict	+	+
	Fehling	+	+
Saponins	Froth Test	+	-
Phytosterols	Salkowsky	+	+
	Liberman	+	+
Phenols	Ferric Chloride	-	-
Tannins	Gelatin	-	+
Flavonoids	Alkaline Reagent	+	+
	Lead acetate	+	+
Proteins and amino acids	Ninhydrin	-	+
Diterpenes	Copper acetate	+	+

(+) Present , (-) Absent

Many natural compounds are found in the *Ephedra altissima* plant, when the extraction procedure previously described was applied. The findings displayed in Table (10), confirmed that, the E.altissima plant contains; alkaloids, proteins, carbohydrates, saponins, flavonoids, Tannins, Phytosterols, and terpenes.

The findings aligned with the results of Salem Edrah and colleagues' investigation, which identified the presence of saponins, cardiac glycosides, flavonoids, steroids, and terpenes (18). Likewise, the results compatible with phytochemical analysis conducted by Mezogi and colleagues, which revealed the presence of alkaloids, flavonoids, terpenoids, carbohydrates, and saponins in the extracts of *Ephedra altissima's* stem (19).

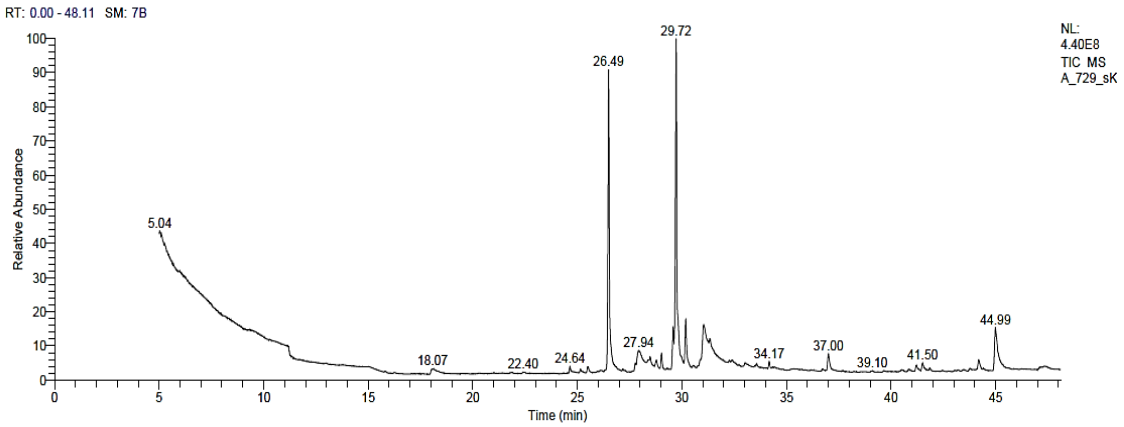
These results are significant, especially in light of the fact that these compounds have a variety of biological functions, including antioxidant, antiviral, and antifungal properties (20).

Chemical analysis of the studied plant showed presence of the natural compounds like: alkaloids, proteins, carbohydrates, saponins, flavonoids, Phytosterols, Tannins,and terpenes.

The aqueous extract showed the presence of compounds: alkaloids, proteins, carbohydrates, saponins, flavonoids, Phytosterols, and terpenes, while phenol and tannins are absent. Whereas the ethyl acetate extract showed the presence of: alkaloids, proteins, carbohydrates, saponins, flavonoids, phytosterols, tannins and terpeneswith absence of phenol.

6- (GC-MS) analysis of *Ephedra altissima*

GC-MS analysis of the extract of the aerial parts of the *Ephedra altissima* plant revealed the spectrum shown in the **figure (3).** Some of the compounds were shown in the **table (4)**



RT	Area %	Peak Area	Peak Height
5.05	0.37	18725864.23	5329403.71
5.10	0.50	25312126.63	5997664.76
11.19	2.08	105622297.97	8300703.51
18.07	0.88	44477421.58	4543861.35
24.64	0.68	34396537.43	8078731.49
25.15	0.41	21037394.06	4411786.55
25.50	0.73	36934547.73	7150640.16
26.49	28.76	1461810992.31	353884403.95
27.76	0.32	16427728.57	5199584.22
27.94	2.89	146787081.58	14558771.72
28.48	1.22	62139454.07	8565786.77
28.77	0.89	45358144.52	8070143.13
29.02	1.56	79491339.72	18587520.10
29.57	2.07	105466984.34	32079359.54
29.72	34.74	1765788692.63	381367591.39
30.18	4.63	235100855.14	52607506.55
31.03	4.69	238472267.01	30579874.33
31.34	0.72	36797445.84	7518205.81
32.40	0.86	43774594.01	4636332.08
33.57	0.52	26601618.31	4106713.08
34.17	0.63	32255725.46	8407888.64
37.00	1.93	98184645.82	18767840.52
41.20	0.69	35241708.53	6333262.12
41.50	0.87	44319417.73	8037612.49
44.19	1.18	60194146.17	10710476.96
44.99	5.16	262048771.55	41757212.90

Figure 3: Full GC Chromatogram of extract of *Ephedra altissima*

Table 1: Compounds found in *Ephedra altissima* plant using (GC/MS)

No	RT	Compound name	Area%	M.F	M.wt
1	26.49	Hexadecanoic acid, methyl ester	28.76	C17H34O2	270
2	27.49	n-Hexadecanoic acid	2.89	C16H32O2	256
3	28.48	Pentadecanoic acid	1.22	C15H30O2	242
4	29.02	2-Acetoxy-1,1,10-trimethyl-6,9-epi dioxydecalin	1.56	C15H24O4	268
5	29.57	(E,E)-9,12-Octadecadienoic aci d, Methyl ester,	2.07	C19H34O2	294

6	29.72	Hexadecatrienoic acid, Methyl ester	34.74	C17H28O2	264
7	30.18	Octydecenoic acid methyl ester	4.63	C19H38O2	298
8	31.03	9-Octadecenoic acid (z)-	4.69	C18H34O2	282
9	31.34	(z,z)-9,12-Octadecadienoic acid	0.72	C18H32O2	280
10	37.00	3',8,8'-Trimethoxy-3-piperidyl-2,2'-b inaphthalene-1,1',4,4'-tetrone	1.93	C28H25NO7	487
11	41.20	à-d-Xylopyranoside, methyl-2,3,4-tris-O-[9-borabicyclo[3 .3.1]non-9-yl]-	0.69	C30H51B3O5	524
12	44.19	Cholesta-8,24-dien-3u-OL, 4-methyl-, (3á,4à)-	1.18	C28H46O	398
13	44.99	Vitamin E	5.16	C29H50O2	430

RT: Retention time

The methanolic extract of the aerial parts of Ephedra altissima primarily consists of several key components. The main constituents include hexadecatrienoic acid, methyl ester (34.74%) and hexadecanoic acid, methyl ester (28.76%). Additionally, there are various low-concentration compounds such as 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol and Vitamin E (5.16%), as well as 9-octadecenoic acid (z)- (4.69%) and n-hexadecanoic acid (2.89%). These findings suggest that the aerial parts of Ephedra altissima are a rich source of bioactive compounds.

The investigated plant exhibits a diverse array of chemical constituents in both quality and quantity, which may contribute to its potential biological activity.

The results of this study demonstrate that the plant examined is a significant source of bioactive compounds, which are frequently utilized in phytotherapy for the treatment of various diseases. Hexadecanoic acid, a physiologically active compound, is primarily employed in the management of infections (21). Additionally, vitamin E, a lipid-soluble antioxidant located at the membrane level, inhibits numerous lipid peroxidation processes (22). Vitamin E interacts with lipid radicals, mitigating their propagation by chelating them and transforming them into less reactive free radicals compared to peroxyl radicals (•LOO) (23). Phytosterols are well-documented for their biological and therapeutic properties. Among these, stigmasterol is particularly noted for its cholesterol-lowering, anticancer, and antioxidant effects. The biological activities of stigmasterol may be attributed to its functional tetracycline rings and the unsaturation of the anthracene group, which exhibit autoxidation capabilities (24).

7- Anti-oxidant analysis

The IC50 value of the samples is determined based on the curves presented in Figures 4 and 5, which illustrate the activity of the investigated samples in inhibiting the free radical DPPH. A lower IC50 value indicates a more effective inhibitory action. This finding demonstrates that the inhibition of the free radical DPPH by the ethanolic extracts of the plant parts under study, as well as by the control ascorbic acid, is directly correlated with increases in concentration, as shown in Tables 5, 6, and 7.

Table 5: The effect of the *Ephedra altissima* extracts on DPPH wall.

Ephedra altissima, µg/ml	OD R1	OD R2	OD R3	OD Mean	DPPH scavenging%	SD	SE
1000	0.095	0.091	0.089	0.092	94.3	0.003	0.001
500	0.145	0.161	0.157	0.154	90.4	0.00	0.00

						8	3
250	0.269	0.270	0.284	0.274	83.0	0.008	0.003
125	0.395	0.389	0.394	0.393	75.7	0.003	0.001
62.5	0.522	0.531	0.541	0.531	67.1	0.010	0.003
31.25	0.642	0.639	0.641	0.641	60.3	0.002	0.000
15.625	0.748	0.751	0.742	0.747	53.7	0.005	0.001
7.8125	0.912	0.921	0.931	0.921	42.9	0.010	0.003
3.9	1.045	1.053	1.051	1.050	35.0	0.004	0.001
1.95	1.167	1.175	1.139	1.160	28.1	0.019	0.006
0	1.62	1.601	1.622	1.614	0.0	0.012	0.004

IC50 =13.9 µg/ml

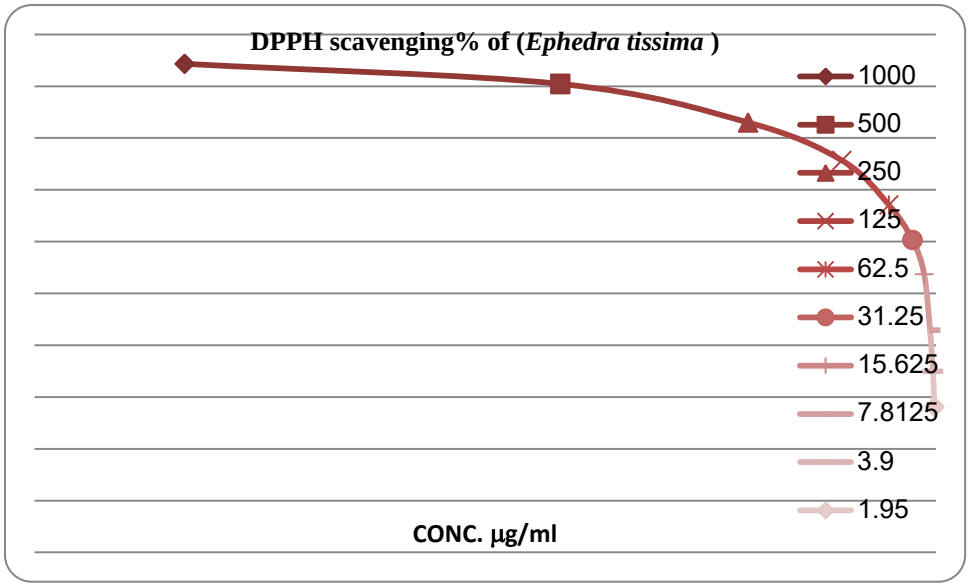


Figure 4: The activity curves for inhibiting DPPH of *Ephedra altissima.*

Table 2: The effect of concentration of Ascorbic acid on DPPH walls

Ascorbic acid. µg/ml	OD R1	OD R2	OD R3	OD Mean	DPPH scavenging%	SD	SE
1000	0.01	0.012	0.017	0.013	99.1	0.004	0.001
500	0.09	0.078	0.081	0.083	94.2	0.006	0.002
250	0.109	0.112	0.117	0.113	92.2	0.004	0.001
125	0.215	0.242	0.235	0.231	84.0	0.014	0.005
62.5	0.371	0.383	0.374	0.376	73.9	0.006	0.002
31.25	0.485	0.491	0.499	0.492	65.9	0.007	0.002
15.625	0.602	0.608	0.611	0.607	57.9	0.005	0.001
7.8125	0.735	0.725	0.741	0.734	49.1	0.008	0.003
3.9	0.851	0.843	0.854	0.849	41.1	0.006	0.002
1.95	0.997	0.957	0.964	0.973	32.5	0.021	0.007
IC50	8.041						

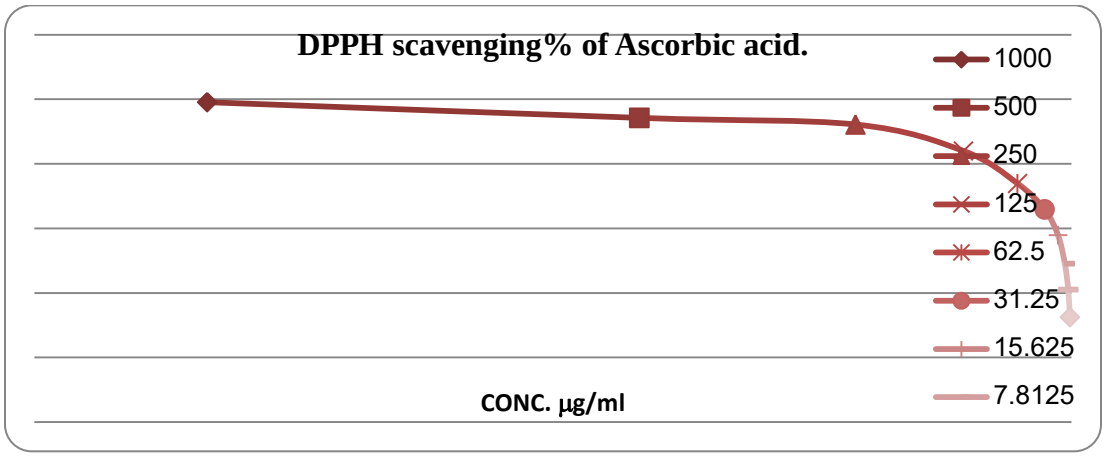


Figure 5: The activity curves for inhibiting DPPH of Ascorbic acid

Table 3: The IC50 values for the studied samples

Sample	Ascorbic acid	*Ephedra altissima*
IC50 ug/ml	8.041	13.49

Plant extracts have been shown to possess the capability to generate hydrogen, which in turn inhibits DPPH radicals, as evidenced by their IC50 values; lower IC50 values indicate stronger antioxidant activity. The data presented in Table 7 reveal that the extract derived from the aerial parts of *Ephedra altissima* exhibits significant antioxidant activity, with an IC50 value of approximately 13.49 µg/mL. In comparison, ascorbic acid, a widely recognized standard for antioxidant activity, demonstrated superior effectiveness. Consequently, it can be concluded that the extract demonstrates commendable antioxidant activity relative to the reference substance..

8- Evaluation of the Efficacy of Extracts Against Bacterial Strains

One objective of this study is to evaluate the antibacterial efficacy of raw plant extracts obtained from *Ephedra altissima* against specific bacterial strains, specifically Escherichia coli and Staphylococcus aureus. The assessment was conducted using filter paper discs, each saturated with the respective extracts. Following saturation, the discs were dried and placed on an agar medium to facilitate bacterial growth for 24 hours at a temperature of 37°C.e of the aims of this study is to assess the antibacterial efficacy of raw plant extract derived from *Ephedra altissima* against specific bacterial strains, namely Escherichia coli and Staphylococcus aureus. The evaluation was performed utilizing filter paper discs, each individually saturated with the respective extracts. After saturation, the discs were dried and subsequently positioned on an agar medium to promote bacterial growth for a period of 24 hours at a temperature of 37°C.

Table 4: The inhibitory effect of studied plants on some bacterial strains

Bacterial strains	**Aqueous extract**	**Et.Ac Extract**	**H_2O**	**Et.Ac**
Escherichia coli	10mm	12 mm	6 mm	7 mm
Staphylococcus aureus	6 mm	10mm	6 mm	7 mm

The results presented in Table 8 indicate that both the aqueous extract and ethyl acetate extract of the investigated plant exhibited significant inhibitory effects against two bacterial species, Escherichia coli and Staphylococcus aureus, following 24 hours of incubation at 37°C. The impact of the various extracts was evidenced by the formation of a clear zone around the extract-infused tablets, referred to as the inhibition zone. This effect was quantified through measurements of the diameters of these inhibitory zones.

The ethyl acetate extract exhibited superior inhibitory activity against the Gram-negative bacterium Escherichia coli, with an inhibition zone diameter of 12 mm, in contrast to the aqueous extract, which demonstrated an inhibition zone of 10 mm. Conversely, the aqueous extract displayed reduced inhibitory effects against the Gram-positive bacterium Staphylococcus aureus, measuring 6 mm in diameter, while the ethyl acetate extract produced a larger inhibition zone of 10 mm.

These findings indicate that the Gram-negative strains were more susceptible to both extracts of the plant, whereas the Gram-positive strain exhibited lower sensitivity to the ethyl acetate extract and showed no sensitivity to the aqueous extract. Ephedra altissima demonstrates reduced sensitivity to Gram-positive bacterial strains when compared to Gram-negative strains. This phenomenon may be attributed to structural differences in their cell walls: Gram-negative bacteria possess two plasma membranes separated by a layer of peptidoglycan, whereas Gram-positive bacteria have a single plasma membrane and a thicker peptidoglycan layer. Consequently, the cell walls of Gram-negative bacteria tend to be thicker than those of their Gram-positive counterparts. This observation supports the hypothesis that the antibacterial

efficacy of phenolic compounds is contingent upon the quantity and spatial arrangement of their hydroxyl groups.

Conclusion

This study has established a significant presence of various phytochemicals, including alkaloids, carbohydrates, saponins, phytosterols, tannins, flavonoids, proteins, and terpenes, in *Ephedra altissima*. This finding underscores the plant's potential importance in medicinal practices and its physiological properties. It is recommended that further phytochemical screenings be conducted utilizing a range of solvents to achieve pure isolation and identification of these compounds.

Moreover, advanced analytical techniques such as gas chromatography-mass spectrometry (GC-MS) revealed a substantial quantity of diverse chemical classes, corroborating findings from previous research. This suggests that *Ephedra altissima* may have beneficial therapeutic effects for various health conditions. The positive outcomes of traditional medicinal applications warrant further exploration into the isolation, purification, and characterization of the bioactive constituents present in the plant.

The DPPH assay performed on the crude extracts of *Ephedra altissima* indicated relatively moderate antioxidant activity compared to the reference compound, ascorbic acid. To gain deeper insights, it is advisable to apply this assay to various solvent extractions and pure isolated compounds.

Finally, the evaluation of antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* revealed variability in efficacy depending on the strain and type of extraction, enhancing the observed inhibitory values. Given the limited studies, confirming the plant's inhibitory effects on these strains, further research employing alternative methodologies and additional bacterial strains is warranted.

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