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The Contents of Fatty Acids, Phenolic Acids, Metals, and Antimicrobial Studies of *Leaves and Fruits of Juniperus Phoenicea* Plant

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ABSTRACT

This study aims to determine of Fatty acids, phenolic acids, minerals, metals, and phytochemical screening in leaves and fruits of the Juniperus phoenicea plant. The extracts are also used for antimicrobial investigation against different species of bacteria. Different methods were used in the experimental part: the GC-MS instrument was used to analyze fatty acids and phenolic compounds, atomic absorption was used to estimate metals, and the spectrophotometer was used to determine phosphorus and Nitrogen in the extracts. The results of this study showed that some phenolic acids were detected in the samples, including: the types of phenolic acids in the leaves and fruits of Juniperus phoenicea were as follows: Juniperus phoenicea leaves: 4,5-Dicaffeoylquinic acid, Cinnamic acid, Gallic acid, Geraniol, Phloridzin, Quercetin, and Catechin. Juniperus phoenicea fruits: 3,4-Dicaffeoly guinic acid, Gallic acid and Catecin. On the other side some of fatty acids were detected and can show as following**:** The saturated fatty acid in leaves and fruits of Juniperus phoenicea as following: Juniperus phoenicea leaves: Hexanoic , Octanoic.Decanoic , Undecanoic ,Dodecanoic , Tridecanoic , Tetradecanoic , Pentadecanoic, Hexadecanoic , Octadecanoic and Eicosanoic Juniperus phoenicea fruits: Decanoic , Undecanoic , Decanoic , Tetradecanoic , Hexadecanoic and Octadecanoic .The high concentration of saturated fatty acid was recorded for Octadecanoic ,while the low concentration was recorded for Decanoic and Undecanoic in fruits of Juniperus pheonicea; and Octanoic in fruits of Pistacia lentiscus**.** The contents of metals were fluctuated as follows: the contents of Na, K, Ca P, and N were as follows 1.50, .40, 2.16, 2.40, and 0.02 in leaf samples, respectively; on the other hand, these mineral contents in fruits were 0.41, 14.44, 1.33, 2.41, and 0.11. The contents of metals of Zn, Cu, Cr, and µg in leaves were 012, 0.84, 0.076, and 1.64 ppm, where the contents of the same metals in fruit samples were 0.13, 0.91, 0.095, and 1.89 ppm, respectively. The antimicrobial activities showed different effects of the fruits and leaves of extracts against different species of bacteria, including Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella Pneumoniae, Escherichia coli, and Salmonella typhimurium**.**

Introduction

Libya has a total area of 1,759,540 square km, of which about 90% is the Sahara Desert, with scattered oases. The largest oasis, Alkufra, lies beneath the desert rock and has a huge underground water supply. The rest of the landmass is covered by a semi-desert region with sparse grazing lands for sheep, goats, camels, and cattle, and natural farmland along the Mediterranean coast. On the northwestern plains and in the northeastern highlands, farmers use mainly traditional methods to grow crops such as oranges, olives, almonds, wheat, and grapes. In Libya, there are about 1,825 vascular plant species, of which 134 are endemic. About 450 species are reported to be of medicinal value [1]. Some important plant families are Apiaceae, Asteraceae, Lamiaceae, Poaceae, Fabaceae, Brassicaceae, and Abiaceae. Medicinal plants are distributed all over the country, especially in the Al-Jabel Al-Akhdar, Ghadames, Gharian, Awbari, and Tarhona regions [2]. More than 100 species are extensively used by Bedouins and local people in folk medicine drinks or chewed fresh or dry. They are used to cure dermal diseases, viral or bacterial infections, insect or animal bites, burns, and sometimes to treat hair problems. These medicinal plants are very well documented in different floras [3]. Many species of medicinal plants, such as *Cupressus sempervirens L., Pinus halepensis Mill., Juniperus phoenicea L., Quercus coccifera L., Asperula arvensis L., Tribulus longipetalus Viv., Veronica cymbalaria Bodard, and Vahlia dichotoma* (Murray) Kuntze, are threatened because of over-harvesting and diversion of forest land to agriculture [4]. There is an urgent need to initiate programmes for the collection and conservation of endangered and rare plant species to save them from extinction as a result of heavy grazing, human use, and drought hazards that occur with increasing frequency. The most famous medicinal plant of Libya is Silphium cyrenaicum (now extinct). It existed during

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Greek and Roman times (900 to 100 B.C.). It was used for the treatment of many illnesses and was so important to the economy that it was sold by weight with silver or gold, and it was depicted on coins [2]. It has been reported that Silphium grew abundantly in Cyrenacia (Al-Jabel Al- Akhdar region), but heavy exploitation led to its extinction hundreds of years ago. The herbal medicines most in demand are chamomile, thyme, and rosemary. Libya exports medicinal plants to Egypt. Trade is handled by the private sector. About 30% of the population relies on traditional medicine in Libya. The Ministry of Health is planning to establish herbal medicine clinics as well as good manufacturing practices in the production of herbal medicinal products, which are mostly imported from Italy and other European countries. There is a lack of information on the formal trade of medicinal and aromatic plants, but during the last twenty years, many chemical and biological studies on the plant constituents were carried out in Libya [5-34]. The results of these studies declared that the plants in Libya contain important amounts of natural product compounds, minerals, and have biological activities. This study aims to estimate the contents of Fatty acids, phenolic acids, minerals, metals, and phytochemical screening, also to evaluate the effect of extracts against different species of pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella typhimurium.*

Methods

Sampling

Selection of medicinal plants for this study

The *Juniperus phoenicea.*plant samples were collected from Al-Gabel Al–Kadar Region. The Samples included leaves and Fruits of *Juniperus phoenicea.*

Samples preparation

Leaves and fruits were separated and washed with distilled water several times, then dried in the open air for fifteen days. Then the samples were ground, and stored until the analysis (Figures 1& 2).

Figure 1. Juniperus phoenicea leaves

Figure 2. Juniperus phoenicea fruits

Taxonomical investigation

The samples were kindly identified by the Plant Taxonomy of the Botany Department, and the samples were kept in the Seliphium herbarium, Faculty of Science, Omar Al Mukhtar University. (Figure 3), Sowa the plant taxonomy.

Phytochemical Screening

It was carried out according to standard methods and previous studies [7-16].

Test for sterols and/or triterpines

l Liebermann-Burchard's test

To 1ml of the chloroformic extract of each preparation, 0.3 ml of acetic anhydride is added, followed by a few drops of concentrated sulphuric acid along the side of the dry test tube. Reddish-violet colour is produced at the junction of the two layers, and chloroformic solution acquires green colour in the presence of sterols and/or triterpenes.

Test for flavonoids

The successive extractives of the tested herbal preparations were further extracted with 1% hydrochloric acid. Each extract was subjected to the following test:10 ml of each extract is rendered alkaline (T.S). Faint yellow colour is produced in case of the presence of flavonoids.

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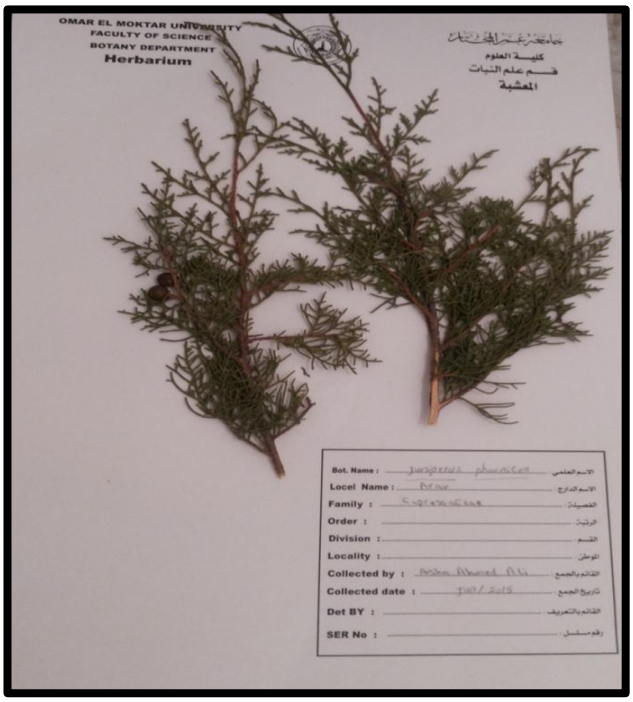

Figure 3. Herbarium sample to Juniperus phoenicea.

Test for alkaloids

The successive extractives of the tested preparations were further extracted with 20 ml of dilute hydrochloric acid, cooled and rendered alkaline with dilute ammonium hydroxide solution, then extracted with chloroform. The chloroformic extract is subjected to the following test:

Dragendorff's test

Preparation of the reagent:

-Solution a: 0.85 g of basic bismuth nitrate is dissolved in a mixture of 10 ml of acetic acid and 40 ml of water. Solution b: 8 g potassium iodide in 20 ml of water. Stock solution: Equal volumes of solutions a and b are mixed. A few drops of chloroformic extract were applied to filter paper, allowed to dry, and sprayed with the reagent. Orange colour is observed in cases of the presence of alkaloids.

Tese for tannins

The successive extraction of the tested herbal preparations was further extracted with ethanol 50%, filtered, and the hydro-alcoholic clear solution was subjected to the following test:

Ferric chloride test

Preparation of the reagent: 1 g of ferric chloride is dissolved in 100 ml of water. 1ml of the reagent is added to the hydro-alcoholic solution. Blue color develops in cases of the presence of pyrogallol tannins.

Test for carbohydrates and /or glycosides

The successive extractives of the tested herbal preparations were further extracted with water, and the produced aqueous extract was subjected to the Molish test.

Molish test

2ml of the aqueous extract is mixed with 0.2 ml ethanolic α-naphthol (20%), and 2ml of concentrated sulphuric acid is added to the side of the dry test tube. A violet ring is observed at the junction of the two layers, indicating the presence of carbohydrates and/or glycosides.

Tests for cardiac glycosides

Keller-Killiani test

1 ml of each extract of the successive extracts of the tested herbal preparations was dissolved in glacial acetic acid containing traces of ferric chloride; concentrated sulphuric acid containing the same amount of ferric chloride was placed at the bottom of the test tube with a pipette. Intense blue colour at the surface between the reagents develops in 2-5 minutes, spreading gradually into the acetic acid layer in cases of the presence of deoxy-sugars.

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Kedde's test
To 1 ml of each extract of the successive extracts of the tested herbal preparations 0.5 ml of Kedd's reagent(3,5-dinitrobenzoic acid solution) is added, followed by 1 ml of ethanolic solution of sodium hydroxide. Purple colour is formed in cases of the presence of cardinolides.

Test for anthraquinones
Bornträger's test
To 1ml of each extract of the successive extracts, aqueous ammonia or caustic soda is added and shaken. Rose-red colour in the aqueous layer develops in cases of the presence of anthraquinone glycosides.

Modified-Bornträger's test
1ml of each extract of the successive extracts of the tested herbal preparations is hydrolyzed with alcoholic potassium hydroxide, acidified, and continues as Bornträger's test. Rose-Red developed in the aqueous layer in cases of the presence of anthraquinones.

Test for saponins
Froth test: 5 ml of tap water is added to 1 ml of each extract, shaken vigorously for five minutes, and the froth develops to a height of 1 cm and persists for 15 minutes, indicating the presence of saponins.

Chemical studies
Total phenolic content (TPC)
Total phenolic was estimated using the colorimetric method based on Folin-Ciocalteu reagent. "100,200,300,400,500µl" of methanol extract of leaves and fruits of the selection plant were diluted by 2ml of distilled water and mixed with "600µl" of Folin-Ciocalteu reagent. The mixture was allowed to stand for 5 min. And then 2ml of 20% Na_2CO_3 was added and kept at a boiling water bath for 1 minute, after which the blue color formed was measured at 765 nm by a UV-visible spectrophotometer. Quantification was done with respect to the standard calibration curve of Pyrogallol, the results were expressed as pyrogallol "µg/ml".

Fatty acids (Gas Liquid Chromatographic Analysis)
5 grams of powdered extract was for 30 minutes with 20ml mixture of chloroform and methanol (2:1) and filtered. The marc (remained powdered) was extracted three times as mentioned (chloroform/ methanol). Combine the extracts and wash with distilled water. The extracted layer was concentrated into a residue. The analysis of fatty acids was carried out by a Shimadzu-8A GLC, in the Faculty of Science, Alexandria University, Egypt.

Determination of minerals
The mineral content of the samples, Na, K, and Ca, were measured by a Flam photometer, while the metals of Mg, Cu, Zn, and Cr were determined by atomic absorption, whereas the P and N were estimated by Spectrophotometer according to the method described by previous studies After digestion with HNO_3 and $HClO_4$ acid**,** were determined using Atomic Absorption Spectrophotometer. These methods were used to estimate minerals and metals in solid and liquid samples [35-40]

Antimicrobial activity
Preparation extract
The whole aerial part of the plants collected was identified. The plant was then dried in the shade and reduced to coarse powder using a mechanical grinder. The powdered plant (100g) was extracted for 72h with methanol 80 % using a rotary evaporator and stored until further use.

Microorganisms
The extracts were individually tested against pathogenic bacteria and fungi. The following bacteria and fungi were tested:

Bacterial strain
Gram-positive bacteria
Bacillus subtilis, Staphylococcus aureus

Gram-negative bacteria
Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli, Klebsiella pneumoniae.

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MIC Determination

The antimicrobial activity of the plant extracts was determined using the agar well diffusion bioassay method. Nutrient agar plates were seeded with the bacterial strain, and Sabouraud dextrose agar plates were seeded with the fungal strain. On each plate, wells were made by a sterile standard cork borer. Each well was filled with 50µl of the different concentrations of studied plant extracts, and the plates were then incubated for a further 24 h at 37°C. The diameters of zones of inhibition were measured. The results are presented as the mean of three independent experiments. The minimal inhibition concentration (MIC) values were evaluated according to published procedures [20-25]. The minimal inhibitory concentration (MIC) was determined only with microorganisms that displayed inhibitory zones. MIC was determined by dilution of the plant extracts and pipetting 50µl of each dilution into wells. Dilutions of the extracts within a concentration range of 0.8- 0.00001 g/ml were also carried out MIC was defined as the lowest concentration that inhibited the visible microbial growth [41].

Results

Preliminary Phytochemical Studies

The dried powdered plants were screened for the following constituents: carbohydrates and/or glycosides, tannins, flavonoids, sterols and/or triterpenes, saponins, and anthraquinone. The obtained results were recorded in (Table 1) and revealed the presence of carbohydrates and/or glycosides, tannins, sterols and/or triterpenes, alkaloids, and cardiac glycosides in all plants, while saponins were absent. The flavonoids are present in all plants, except the anthraquinones, which are absent in all extracts.

Table 1. Phytochemical screening of the studied plants

Plants **Chemical test**	***J.phoenicea***			
	Leaves		Fruits	
	Al	Aq	Al	Aq
Saponins	_	_	_	_
Tannines	+	+	+	+
Carbohydrate and/or Glycosides	+	+	+	+
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Anthraquinones	_	_	_	_
Steroids and/or triterpenoids	/	+	/	+
Cardiac glycosides	/	+	/	+

(+): Present, (-): Absent, (/): Don't done.

Chemical studies

Total phenolic acid content

Phenolic acid content in the studied plant was given in (Table 2) and (Figure 4). The concentration of phenolic acids in leaves and fruits of *Juniperus phoenicea* is as follows: *Juniperus phoenicea* leaves:4,5-Dicaffeoly guinic acid (0.003047 ppm), Cinnamic acid (0.00000696 ppm), Gallicacid (0.0161ppm), Geraniol (0.000644 ppm), Phloridzin (0.00000297 ppm), Quercetin (0.02033 ppm) and Catecin (0.0424 ppm). *Juniperus phoenicea* fruits: 3,4-Dicaffeoly guinic acid (0.00115 ppm), Gallic acid (0.0000975 ppm and Catechin (0.0424 ppm).

Table 2. Phenolic acid contents* ppm *in Juniperus phoenicea plant

Phenolic acids **ppm**	***J. phoenicea***	
	Leaves	Fruits
Chlorogenic acid	_	_
Caffeic acid	_	_
3,4-Dicaffeoyl guinic acid	_	0.00115
3,5-Dicaffeoyl guinic acid	_	_
4,5-Dicaffeoyl guinic acid	0.003047	_
2,5-dihydroxy Benzoic acid	_	_
Cinnamic acid	0.00000698	_
Gallicacid	0.0161	0.0000975
Geraniol	0.000644	_
Phloridzin	0.00000297	_
Quercetin	0.02033	_
Catecin	0.0424	0.0083

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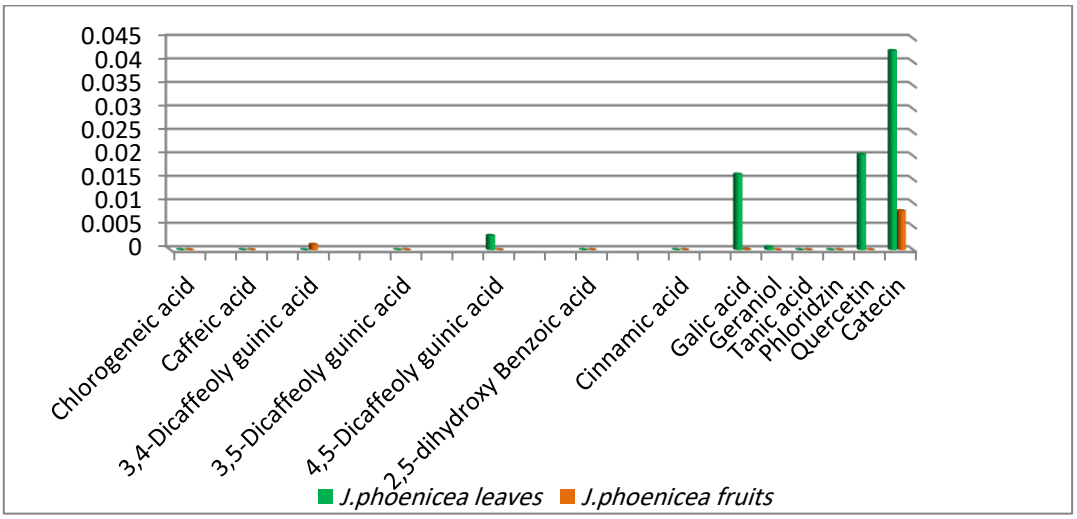

Figure 4. Phenolic acid content in Juniperus phoenicea leaves and fruits.

Fatty acids

Total Saturated and Unsaturated Fatty Acids

Total fatty acid content. The concentration of Saturated fatty acids in *J. phoenicea* leaves and fruits is as follows (0.157 and 0.121 ppm) respectively. Concentration of unsaturated fatty acid in *J. phoenicea* leaves and fruits is as follows: Monounsaturated fatty acid (0.025and 0.046 ppm), respectively. Polyunsaturated fatty acid (0.014 and 0.045ppm), respectively, (Table 3).

Table 3. Total Saturated (T SFA) and unsaturated (Un SFA) fatty acids

T SFA and T UnSFA ppm		***J.phoenicea***	
		Leaves	Fruits
SFA		0.157	0.121
Un SFA	MUFA	0.025	0.046
	PUFA	0.014	0.045

Saturated fatty acids

The concentration of saturated fatty acid in leaves and fruits of *Juniperus phoenicea* as following: *Juniperus phoenicea* leaves: Hexanoic (0.001 ppm), Octanoic (0.002ppm), Decanoic (0.005ppm), Undecanoic (0.008ppm), Dodecanoic (0.029ppm), Tridecanoic (0.011ppm), Tetradecanoic (0.014ppm), pentadecanoic (0.031ppm), Hexadecanoic (0.015ppm), Octadecanoic (0.021ppm), and Eicosanoic (0.020 ppm). *Juniperus phoenicea* fruits: Decanoic (0.001ppm), Undecanoic (0.001ppm), Decanoic (0.005ppm), Tetradecanoic (0.011ppm), Hexadecanoic (0.035ppm), and Octadecanoic (0.068ppm). The high concentration of saturated fatty acid was recorded for Octadecanoic (0.074 ppm) in fruits of Juniperus pheonicea, while the low concentration was recorded for Decanoic and Undecanoic (0.001ppm) in fruits of *Juniperus pheonicea*; and Octanoic (0.001 ppm) in fruits of Pistacia *lentiscus* (Table 4) and (Figure 5).

Table 4. Saturated fatty acid content in the studied plant (leaves and fruits).

Fatty acids ppm	***J.phoenicea***	
	Leaves	Fruits
Hexanoic	0.001	_
Octanoic	0.002	_
Decanoic	0.005	0.001
Undecanoic	0.008	0.001
Dodecanoic	0.029	0.005
Tridecanoic	0.011	
Tetradecanoic	0.014	0.011
pentadecanoic	0.031	_
Hexadecanoic	0.015	0.035
Octadecanoic	0.021	0.068
Eicosanoic	0.020	_

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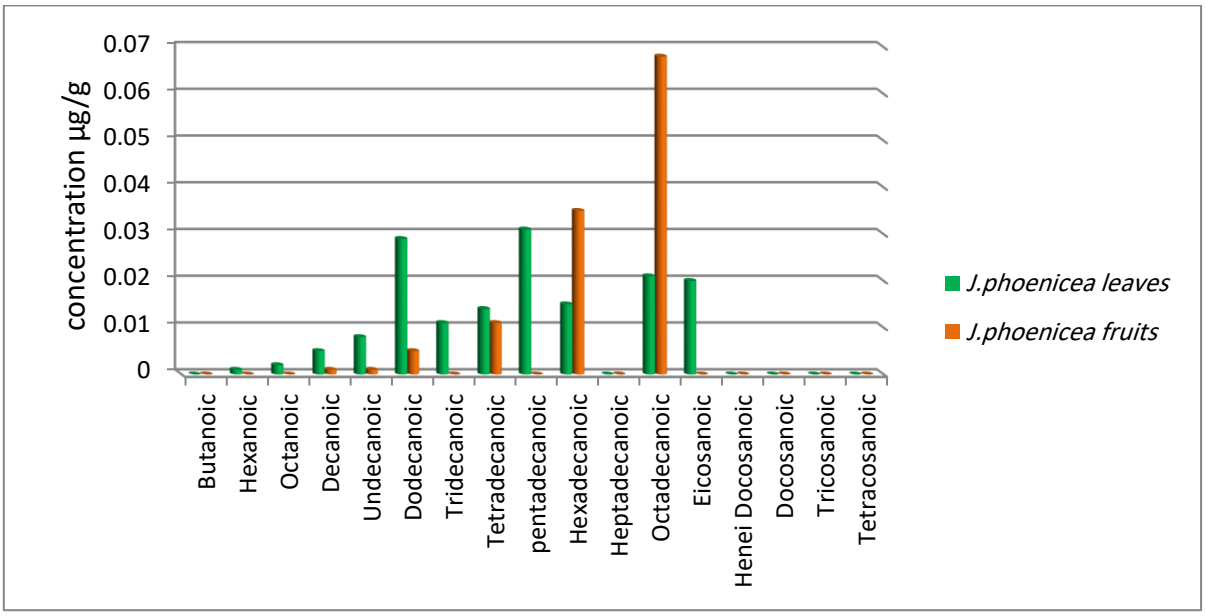

Figure 5. Saturated fatty acid content in J.phoenicea leaves and fruits.

Unsaturated fatty acids

The concentration of unsaturated fatty acids in leaves and fruits of *the studied plant* is as follows: leaves: Myristoleic (0.010 ppm), Oleic (0.015 ppm), and ɣ-linoleic (0.004ppm). At the same time, the same Fatty acids in Fruits showed the contents of Nd, 0.046 and 0.045, respectively, (Table 5).

Table 5. Unsaturated fatty acid content in the studied plant (leaves and fruits).

Fatty acids ppm		***J.phoenicea***	
		Leaves	Fruits
Mono fatty acid	Myristoleic	0.010	_
	Oleic	0.015	0.046
Polyunsaturated fatty acid	ɣ -linoleic	0.014	0.045

Mineral element contents of the Leaves and fruits of the studied Plants

The mineral element constituents of the studied plants are shown in (Table 6). The concentration of macro elements. The high contents of potassium were recorded in leaves and Fruit, with values of 77.4 and 14.4 ppm, respectively. The contents of sodium, calcium, phosphorus, and Nitrogen in leaves were as follows: 1.50, 2.16, 2.40, and 0.02 ppm, whereas their contents in Fruits were 0.41, 1.33, 2.41, and 0.11 ppm, respectively. The contents of Zn, Cu, Cr, and Mg in the fruits were as follows: 0.134, 0.913, 0.0954, and 1.899 ppm, respectively, while their contents in leaves were 0.127, 0.849, 0.076, and 1.647 ppm, respectively (Table 6).

Table 6. Mineral contents of Leaves and fruits of studied plants (ppm)

Plants Elements Ppm		*J.Phoenicea*	
		Leaves	Fruits
Macro Elements	Na	1.50	0.41
	Ca	2.16	1.33
	K	7.4	14.4
	P	2.40	2.415
	N	0.02	0.11
Microelements	Zn	0.127	0.134
	Cu	0.849	0.913
	Cr	0.076	0.0954
	Mg	1.647	1.899

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Antimicrobial activity

In (Tables 7& 8) different concentrations of studied plant extract against *staphylococcus aureus Bacillus subtilis* and *pseudomonas aeruginosa* gave inhibition zone and MIC in all extract, The effect on extracts was differ from concentration to another, where the high values of inhibition zone was obtained at concentration of 0.8 g /ml on the studied bacteria species for the both extracts of inhibition zone values of (30, 21) for leaves and fruit extracts on *staphylococcus aureus*, for the inhibition zone of the effected of extracts on Bacillus the results showd 24 and 25 mm, On the side only leaves extracts showed inhibition zone of 21 mm on the pseudomonas species. The results also did not show an antibacterial effect of concentrations below 0.01 g/ml on the studied bacterial species. The results also do not give the antibacterial effect of the fruit extracts on the Klebsilla, *E. Coli,* and *Salmonella.*

Table 7. Antimicrobial activities of different concentrations of the studied plant extract against Staphylococcus aureus, Bacillus subtilis, and Pseudomonas aeruginosa

Samples Concentration	***Staphylococcus Aureus***		***Bacillus Subtilis.***		***Pseudomonas Aeruginosa***	
	Leaves	**Fruits**	**Leaves**	**Fruits**	**Leaves**	**Fruits**
0.8g/Ml	30	21	24	25	21	N.A
0.4g/Ml	28	16	23	23	20	N.A
0.2 G/Ml	27	13	21	22	17	N.A
0.1 G/Ml	24	11	20	20	14	N.A
0.01 G/Ml	N.A	N.A	N.A	N.A	11	N.A
0.001 G/Ml	N.A	N.A	N.A	N.A	N.A	N.A
0.0001g/Ml	N.A	N.A	N.A	N.A	N.A	N.A

Table 8. Antimicrobial activities of different concentration of the studied plant extracts against Klebsiella pneumoniae, Escherichia coli and Salmonella typhimurium

Samles Con.Cen	***Klebsiella Pneumoniae***		***Escherichia Coli***		***Salmonella Typhimurium***	
	Leaves	Fruits	Leaves	Fruits	Leaves	Fruits
0.8g/Ml	23	N.A	27	24	27	N.A
0.4g/Ml	22	N.A	24	20	24	N.A
0.2 G/Ml	21	N.A	21	12	21	N.A
0.1 G/Ml	16	N.A	20	10	17	N.A
0.01 G/Ml	N.A	N.A	8	9	N.A	N.A
0.001g/Ml	N.A	N.A	N.A	N.A	N.A	N.A

Discussion

In this study, the results showed the presence of different types of Fatty and phenolic acids, besides many natural product compounds. The results also recorded the presence of different types of macro and minerals in both fruits and leaves. The results also showed the presence of effects of the studied extracts on different species of bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas Aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli,* and *Salmonella typhimurium***.** Medicinal plants have been used for centuries as remedies for human diseases, because they contain chemical components of therapeutic value [42]. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [43]. Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phototherapy, spices, and nutrition [44]. Also, the Essential oils are used in traditional medicine for their antiseptic action and constitute 1% of plant secondary metabolites, and are mainly represented by terpenoids, phenypropanoids or benzenoids, fatty acid derivatives, and amino-acid derivatives [45]. The estimate the contents of fatty acid and phenolic acids by modern methods as GC-Mass is very important, because these method scan detect very low concentration s of the samples, also to atomic absorption instrument can estimate low levels of heavy metals [46 -93] The antibacterial activities of the plant extracts are mainly depended on the natural products compound presence in them, the effect of plant extracts on the bacteria halls is give them their antibacterial activities, also some studies recorded the presence in the extracts may be anti-bacterial activities.

Conclusion

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According to the results recorded in this study, the leaves showed antibacterial activities higher than the fruits of the same plant. Also, the results revealed the presence of different amounts and types of Fatty acids and phenolic acids, besides the presence of important amounts of elements as potassium, sodium, calcium, phosphorus, and Nitrogen.

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Conflict
No conflict between the results recoded in this study with other studies.

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