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Original article

Analysis of Fatty Acids, Phenolic Compounds, Antimicrobial Activity, Paper Chromatography, and Metal and Mineral Content of the *Arbutus pavarii* Plant

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Abstract
The contents of fatty acids, phenolic acids, minerals, and some metals, as well as antibacterial activities, were studied in leaf and fruit samples collected from the Al Gabal Al Alakhder area. Different instruments were used in this study. The GC-MS was employed to determine the types and contents of fatty acids and phenolic acids. Atomic absorption spectroscopy was used to measure the concentrations of heavy metals, while the flame photometer was used to estimate the concentrations of potassium, sodium, and calcium. also, the spectrophotometer was applied to measure the contents of phosphorus. The antibacterial activities were carried out on different bacterial species, including positive and Gram-negative bacteria such as Staphylococcus *aureus*, *Bacillus subtillus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella typhimurium.* The results of this study recorded the presence of different types and contents of phenolic acids, including The Phenolic acids content in the studied plant leaves and fruits of *Arbutus pavarii* were ranged as follows: *Arbutus pavarii* leaves: Chlorogenic acid (0.0179 mg/g), 3,4-Dicaffeoylquinic acid (0.1022mg/g), 3,5-Dicaffeoylquinic acid (0.1325 mg/g), and 4,5-Dicaffeoylquinic acid (0.0205 mg/g). For the *Arbutus pavarii* fruits: Chlorogeneic acid (0. .0000026 mg/g), 3,4-Dicaffeoly guinic acid (0.00344 mg/g), 3,5-Dicaffeoly guinic acid (0.00572mg/g), 4,5-Dicaffeoly guinic acid (0.00419mg/g), 2,5-dihydroxy Benzoic acid (0.00000365mg/g), Cinnamic acid (0.00000696 mg/g), Galic acid (0.000014 mg/g) and Geraniol (0.00000314 mg/g). On the other side, the concentrations of fatty acids were the concentrations of Saturated fatty acids in *A.pavarii* leaves and fruits as follows (0.079 and 0.153 mg/g) respectively. Concentration of unsaturated fatty acid in *A.pavarii* leaves and fruits as follows: Monounsaturated fatty acid (0.065 and 0.000 mg/g), respectively. Polyunsaturated fatty acid (0.034 and 0.000 mg/g), respectively. The concentration of saturated fatty acids in leaves and fruits of *Arbutus pavarii* is as follows: *Arbutus pavarii* leaves, Hexadecanoic 0.034 mg/g and Octadecanoic (0.045 mg/g). *Arbutus pavarii* fruits: Butanoic (0.003mg/g), Octanoic (0.007mg/g), Decanoic (0.005mg/g), Tridecanoic (0.073mg/g), and Pentadecanoic (0.011mg/g). The antibacterial activity against the selected bacterial species showed high efficiency of the *Arbutus pavarii* plant extract in the inhibition of most species.
Keywords. Fatty Acids, Phenolic Acids, Minerals, Metals, *Arbutus pavarii* Extract.

Introduction

The universal role of plants in the treatment of diseases is exemplified by their employment in major syfruits of medicine, irrespective of their underlying philosophical premise [1]. The medicinal plants, as an endangered component of biodiversity, received special attention [2]. The medicinal plants are now considered within the global biodiversity strategy. They are termed the “sleeping giant” and will continue to be an important source of drugs because they are manufactured inexpensively and are a source of new products that are seemingly inexhaustible [3]. Libya supported one of the oldest civilizations in the world and has a long history of intense human occupation. Doubtless, the Libyans, through successive eras, depended on plants for food, fuel, fibers, constructions, and folk medicine [4].

Indigenous people (natives) in the region of the eastern Mediterranean coast of Libya tend to be dependent on medicinal plants and often possess exceptional medicinal plant knowledge. However, exposure to modern culture, increased trade, and access to modern conveniences (including modern medicines) are altering the distribution and extent of local knowledge and use of medicinal plants in these societies. Explicitly >Libya has a tremendous wealth of medicinal plants scattered all over a vast area. These plants are used in Libyan folklore medicine for their medical as well as nutritive values [5]. The analysis of many medicinal plants and chemical constituents with tier applications was investigated in many studies during the last twenty years in Libya, most of these concluded that the endemic plants grow in many Libyan regions containing many different important compounds [6-36]. Also, the study of the contents of minerals and metals was investigated in different samples in many studies [37-66]. This study was designed to obtain information regarding the following aspects: phytochemical screening of leaves and fruits.

Chromatography analysis of important compounds of leaves and fruits. Definition and determination of phenolic compounds of leaves and fruits. Definition and determination: Fatty acid compounds of leaves and fruits of the studied plants. Determination of Mineral elements and total nitrogen content of leaves and fruits. Biological and Comparative study between leaves and fruits as antifungal and antibacterial.

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Methods

The Area of Study

The study area is located on AL-Jabal Al-Akhder in Libya, at Mountain locations around El-Beida city. Al Jabal Al Akhdar is a single plateau 700 to 870 meters above sea level with a nundu lating surface that slightly slopes to the south and runs between longitudes 20°, 35′ E to 23°,15′ E and latitudes 30°,58′ N to 32°56′ N. The El-Jabal Al Akhdar area possesses unique physiographic and climatic conditions that provide an excellent ecological niche and contribute to the restriction of many plant species.

Sampling

Selection of medicinal plants for this study

The *Arbutus pavarii* plant samples were collected from Al-Gabel Al–Kadar Region during the Winter Season. The-Samples including the leaves and fruits of *Arbutus pavarii.*

Arbutus pavarii

Taxonomical position of Arbutus pavarii

Kingdom: Plantae, (unranked): Angiosperms, (unranked): Eudicots, (unranked): Asterids, order: Ericales, Family: Ericaceae, Genus: Arbutus, Species: A.pavarii, Binomial name Arbutus pavarii, pampan (Wikipedia, the free encyclopedia 2015).

Samples preparation

Leaves and fruits were separated and washed with distilled water several times, then dried in the open air for fifteen days. After that, the samples were ground by mortar and kept until analysis.

Chromatographic investigation

Materials for paper chromatographic studies

Sheets of Wathmann filter paper No.1.
Chromatographic glaaa tanks.

Solvent syfruits

Acetic acid- Water (15:85 v/v). Methylen chloride- Methanol- Water (60 35 5 v/v)., Benzene-Ethyl acetate-Acetic acid (12:4:0.5 v/v).
spray reagents: Ferric chloride for phenolic compounds, 1% Ferric chloride solution in ethanol, yields a blue to green color. Aluminum chloride for flavonoids, Aluminum chloride solution in ethanol yields yellow fluorescence. Potassium hydroxide for anthraquinone**,** Potassium hydroxide solution, ethanol yields pink.

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 methanolic 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 cooling, the blue colour formed was measured at 765 nm by UV-visible spectrophotometer. Quantification was done with respect to a standard calibration curve of Pyrogallol, the results were expressed as pyrogallol "µg/ml" [14].

Fatty acids (Gas Liquid Chromatographic Analysis)

5 grams of powdered extract for 30 minutes with 20ml mixture of chloroform and methanol (2:1), and filtered. The marc (remained powdered) re re-extracted three times as mentioned (chloroform/ methanol). Combine the extracts and wash with distilled water. The extracted layer was concentrated to a residue**.** The analysis of fatty acids carried out by a Shimadzu-8A GLC in the Faculty of Science, Alexandria University, Egypt [6].

Determination of minerals

The mineral content of the samples, i.e., Na, K, Mg, Ca, Cu, Zn, Mn, Fe, and P, was determined. All minerals, except Na, K, and P, were determined with an atomic absorption Spectrophotometer (Milton Roy Perkin Elmer 3300) according to the method described by previous studies. Soluble sodium and potassium contents were carried out with a Flame Photometer (Flame Photometer) according to the method described by previous studies. Total phosphorus was determined spectrophoto-metrically using the procedure of After digestion with H_2SO_4 and $HClO_4$, acid was determined using an Atomic Absorption Spectrophotometer according to the method described by previous studies [18].

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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.

MIC Determination*:*

The antimicrobial activity of the plant extracts was determined using the agar well diffusion bioassay method [84].

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 the studied ' 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 of some previous studies [85,86]. 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.The antibacterial sensitivities were carried out according some previous studies [7]by comparing the obtained results with some standard antibiotics as amoxicillin, ampicillin, and others [7].

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 samples of aqueous (Aq) and alcoholic (Al) extracts, while saponins and anthraquinones were absent.

Table 1. Phytochemical screening of leaves and the A pavarii plant

Chemical test	***A. pavarii***			
	leaves		**fruits**	
	Aq	Al	Aq	Al
Saponins	_	_	_	_
Tannines	+	+	+	+
Carbohydrate and/or Glycosides	+	+	+	+
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Anthraquinones	_	_	_	_
Steroids and/or triterpenoids	+	/	+	/
Cardiac glycosides	+	/	+	/

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

For the paper chromatography analysis, the obtained results recorded the presence of phenols and flavonoids. This test depends on the separation of these under uses different solvents or solvent syrups (Table 2) and (Figures 1- 9).

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Table 2. Paper chromatographic investigation of alcohol and aqueous extract of Arbutus pavarii leaves and fruits.

Solvent	Reagent	*A. pavarii*			
		Leafs		fruits	
		Al	Aq	Al	Aq
15% acetic acid	$FeCl_3$	Blue Rf=0.33	Blue Rf=0.32	Blue Rf=0.24	Blue Rf=0.21
	$AlCl_3$	Yellow Rf=0.25	Yellow Rf=0.42	Invisible	Invisible
	KOH	Pink Rf=0.24	Yellow Rf=0.25	Yellow Rf=0.42	Invisible
Benzene-Ethyl acetate- Acetic acid (12:4:0.5 v/v)	$FeCl_3$	Blue Rf=0.068	Blue Rf=0.09	D.m	D.m
	$AlCl_3$	Yellow RF=0.03	Yellow Rf=0.05	D.m	Invisible
	KOH	Pink RF=0.09	Pink RF=0.13	D.m	Invisible
Methylene chloride-Methanol-Water (60:35:5 v/v)	$FeCl_3$	Blue Rf=0.84	Blue Rf=0.77	Blue Rf=0.4	D.m
	$AlCl_3$	Yellow Rf=0.81	Yellow Rf=0.76	Yellow Rf=0.34	D.m
	KOH	Pink Rf=0.81	Pink Rf=0.78	Pink Rf=0.13	D.m

Figure 1. Paper chromatographic investigation of alcohol and aqueous extract of Arbutus pavarii leaves and fruits (solvent acetic acid/reagent $AlCl_3$).

Figure 2. Paper chromatographic investigation of alcohol and aqueous extract of Arbutus pavarii leaves and fruits (solvent acetic acid/reagent KOH).

Figure 3. Paper chromatographic investigation of alcohol and aqueous extract of Arbutus pavarii leaves and fruits (solvent Benzene-Ethyl acetate- Acetic acid/reagent $FeCl_3$)

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Figure 4. Paper chromatographic investigation of alcohol and aqueous extract of Arbutus pavarii leaves and fruits (solvent Benzene-Ethyl acetate- Acetic acid/reagent $AlCl_3$).

Figure 5. Paper chromatographic investigation of alcohol and aqueous extract of Arbutus pavarii leaves and fruits (solvent Benzene-Ethyl acetate- Acetic acid/reagent KOH).

Figure 6. Paper chromatographic investigation of alcohol and aqueous extract of Arbutus pavarii leaves and fruits (solvent Methylene Chloride-Methanol-Water / reagent $FeCL_3$).

Figure 7. Paper chromatographic investigation of alcohol and aqueous extract of Arbutus pavarii leaves and fruits (solvent Methylene Chloride-Methanol-Water / reagent $AlCl_3$).

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Figure 8. Paper chromatographic investigation of alcohol and aqueous extract of Arbutus pavarii leaves and fruits (solvent Methylene Chloride-Methanol-Water / reagent KOH).

Chemical studies

Total phenolic acid content

The Phenolic acids content in the studied plant leaves and fruits of *Arbutus pavarii* ranged as follows: *Arbutus pavarii* leaves: Chlorogenic acid (0.0179 mg/g), 3,4-Dicaffeoylquinic acid (0.1022mg/g), 3,5-Dicaffeoylquinic acid (0.1325 mg/g), and 4,5-Dicaffeoylquinic acid (0.0205 mg/g). For the *Arbutus pavarii* fruits: Chlorogeneic acid (0. .0000026 mg/g), 3,4-Dicaffeoly guinic acid (0.00344 mg/g), 3,5-Dicaffeoly guinic acid (0.00572mg/g), 4,5-Dicaffeoly guinic acid (0.00419mg/g), 2,5-dihydroxy Benzoic acid (0.00000365mg/g), Cinnamic acid (0.00000696 mg/g), Galic acid (0.000014 mg/g) and Geraniol (0.00000314 mg/g), (Table 3).

Table 3. Phenolic acid content in Arbutus pavarii (leafs and fruits)

Phenolicacids Mg/g	***A.pavarii***	
	leafs	**fruits**
Chlorogenic acid	0.0179	0.0000026
Caffeic acid	_	_
3,4-Dicaffeoly guinic acid	0.1022	0.00344
3,5-Dicaffeoyl guinic acid	0.1325	0.00572
4,5-Dicaffeoyl guinic acid	0.0205	0.00419
2,5-dihydroxy Benzoic acid	_	0.00000365
Cinnamic acid	_	0.00000696
Galic acid	_	0.000014
Geraniol	_	0.00000314
Tannic acid	_	_
Phloridzin	_	_
Quercetin	_	_

Fatty acids

Total Saturated and Unsaturated Fatty Acids

The total fatty acid content.*Arbutus pavarii*: the concentration of Saturated fatty acids in *A.pavarii* leaves and fruits is as follows (0.079 and 0.153 mg/g), respectively. The concentration of unsaturated fatty acids in *A. Pavarii leaf leaves*and fruits are as follows: Monounsaturated fatty acid (0.065 and 0.000 mg/g), respectively. Polyunsaturated fatty acid (0.034 and 0.000 mg/g), respectively, (Table 4).

Table 4. Total Saturated (T SFA) and unsaturated (Un SFA) fatty acids:

T SFA and T UnSFA mg/g		***A.pavarii***	
		Leafs	**Fruits**
SFA		0.079	0.153
Un SFA	MUFA	0.065	0.000
	PUFA	0.034	0.000

Saturated fatty acids

The concentration of saturated fatty acids in leaves and fruits of *Arbutus pavarii* is as follows: *Arbutus pavarii* leaves Hexadecanoic 0.034 mg/g and Octadecanoic (0.045 mg/g). *Arbutus pavarii* fruits: Butanoic (0.003mg/g), Octanoic (0.007mg/g), Decanoic(0.005mg/g), Tridecanoic (0.073mg/g), and Pentadecanoic (0.011mg/g), (Table 5).

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Table 5. Saturated fatty acid content in the studied plants (leaves and fruits).

Fatty acids mg/g	***A.pavarii***	
	Leafs	**Fruits**
Butanoic	_	0.003
Hexanoic	_	_
Octanoic	_	0.007
Decanoic	_	0.005
Undecanoic	_	_
Dodecanoic	_	_
Tridecanoic	_	0.073
Tetradecanoic	_	_
pentadecanoic	_	0.011
Hexadecanoic	0.034	_
Heptadecanoic	_	_
Octadecanoic	0.045	0.054
Eicosanoic	_	_
Henei Docosanoic	_	_
Docosanoic	_	_
Tricosanoic	_	_
Tetracosanoic	_	_

Unsaturated fatty acids

The concentration of unsaturated fatty acids in the leaves and fruits of *Arbutus pavarii* is as follows: *Arbutus pavarii* leaves: Oleic (0.065 mg/g) and ɣ-linoleic (0.034mg/g). *Arbutus pavarii* fruits don't have any Unsaturated fatty acids.

Mineral element contents of The Leafs and fruits of the studied Plant:

The mineral element constituents of the studied plant are shown in (Table 6). The concentration of macro elements magnesium was the highest in leaves of *Arbutus pavarii,* followed by Copper, zinc, and chromium. Leaves and fruits of *Arbutus pavarii* contained the highest amount of Magnesium (1.984, 1.85 ppm), respectively. The higher concentration of Copper was present in the leaves of *Arbutus pavarii* (1.017 ppm). The Zinc was present in higher concentration in the leaves of *Arbutus pavarii* (0.184 ppm). The Irom was presented in higher concentration in Leafs of *Arbutus pavarii* (0.1732ppm). Sodium was present in higher concentrations in the leaves and fruits of *Arbutus pavarii.* The Calcium was present higher concentration in *Arbutus pavarii* 1.08 ppm), and the Calcium in the fruits of *Arbutus pavarii* (0.83 ppm). The potassium was present in higher concentration in the fruits of *Arbutus pavarii* (13.5 ppm), and the potassium in the leaves of *Arbutus pavarii* (11.1 ppm). The phosphors were present higher concentration in the leaves of *Arbutus pavarii* (2.512 ppm) (Table 7).

Table 6. Mineral contents of leaves and fruits of the studied plant (ppm)

Elements ppm		***A.pavarii***	
		Leafs	**Fruits**
Macroelements	Na	0.51	0.50
	Ca	1.08	0.83
	K	11.1	13.8
	P	2.512	2.343
	N	0.30	0.27
Microelements	Zn	0.184	0.116
	Cu	0.951	0.871
	Cr	0.1732	0.0943
	Mg	1.984	1.851
	Fe		

Antimicrobial activity

Antimicrobial activity of methanol extract of four medicinal plants (leaves and fruits). For the Gram-positive bacteria (*Staphylococcus aureus*), the different concentrations of studied extracts against *Staphylococcus aureus* gave an inhibition zone, and MIC in all extracts was 0.1 g/ml, except *A.pavarii* leaf extracts were 0.01g/ml. For Gram-negative bacteria (*Pseudomonas aeruginosa*), the different concentrations of the studied extract against *Pseudomonas aeruginosa* gave an inhibition zone.MIC in leaves and fruits of *A.pavarii* extract was 0.1 g/ml, and MIC in leaf leaves was 0.01 g/ml. *For Klebsiella pneumoniae,* different concentrations of the studied extract against *Klebsiella pneumoniae* not give an inhibition zone. For *Escherichia coli,* different

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concentrations of the studied plant extract against *Escherichia coli* gave an inhibition zone.MIC in all plants was 0.001 g/ml, MIC in fruits of *A.pavarii* was 0.1g/ml, MIC. For *Salmonella typhimurium***,** different concentrations of the studied plant extract against *Salmonella typhimurium* gave an inhibition zone. MIC in all extracts was 0.1 g/ml (Table 7).

Table 7. Antimicrobial activities of different concentrations of the studied plant extracts against the studied bacterial species.

Samples Concentration	*staphylococcus aureus*		*Bacillus subtillus*		*pseudomonas aeruginosa*		*Klebsiella pneumoniae*		*Eschericha coli*		*Salmonella typhimurium*	
	leafs	**fruits**	**leafs**	**fruits**	**leafs**	**fruits**	**leafs**	**fruits**	**leafs**	**fruits**	**leafs**	**fruits**
0.8g/ml	26	23	25	19	30	25	N.A	N.A	28	20	25	25
0.4g/ml	25	22	23	18	24	22	N.A	N.A	21	18	20	22
0.2 g/ml	16	21	22	15	20	20	N.A	N.A	20	15	16	20
0.1 g/ml	15	20	17	10	17	18	N.A	N.A	15	11	10	19
0.01 g/ml	10	N.A	N.A	N.A	N.A	N.A	N.A	N.A	10	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A	N.A	N.A	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	N.A	N.A	N.A	N.A	N.A	N.A
0.00001g/ml	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A

Discussion

Medicinal plants have been used for centuries as remedies for human diseases, because they contain chemical components of therapeutic value [88]. 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 [67-70]. Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phototherapy, spices, and nutrition [68]. 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 [71-72].

Herbal drugs are still the spine of about 75–80% of the world's population, mainly in developing countries, for the treatment of many diseases due to better compatibility with the human body and producing fewer adverse effects. It has been assessed that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from plant chemical constituents such as Atropine (Anticholinergic), cardiac glycosides (cardiotonics), artimisinin (antimalarial), Opium alkaloids (analgesics), quinine (antiparasitic), taxol (antineoplastic), vincristine and vinblastine (antineoplastic) [73-75].

Herbal plants have wide therapeutic value for a long time, and still a lot of research is going on which further explores their use to improve human health. In this study, the GC-MS was used to estimate the contents of phenolic and fatty acids. This method is very accurate and was used to determine different aromatic and aliphatic compounds in different samples [76-86]. In Libya, many studies concluded that the endemic Libyan plants containing different types of natural products and their extracts showed efficiency against different species of organisms .Also, some studies indicated that the presence of some metals with other complexes may show antibacterial activities [87-90].

Conclusion

Medicinal plants continue to play a vital role in global healthcare, particularly in developing countries, due to their therapeutic efficacy and minimal side effects. The presence of essential oils, phenolic compounds, and bioactive metabolites underscores their pharmacological potential. This study, through GC-MS analysis, confirmed the diversity of aromatic and aliphatic compounds in plant samples, reinforcing the value of Libyan endemic flora in antimicrobial research. Ongoing investigations into their chemical composition and biological activity may pave the way for novel therapeutic applications and support the integration of traditional remedies into modern medicine.

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Conflict

The results recorded in this study are original, and there are no disputes or problems between the researchers or others.

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