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Original Research Article

Chemical Composition, and Antibacterial and Antifungal Activities of Crude Extracts from *Pistacia lentiscus* L. Fruit

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ABSTRACT

Pistacia lentiscus L is a widely distributed species with ethnopharmacological uses. Despite the large body of literature on its anti-inflammatory, antioxidant, and anticancer activities, several aspects of its biological activities remain unexplored. This study aimed to investigate the antimicrobial potential of chloroform and methanol crude extracts of *P. lentiscus* fruit and to characterize the chemical composition of these crude extracts. The microorganisms employed were four bacteria strains, namely,*Staphylococcu aureus*,*Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and one fungus *Candida albicans*. The susceptibility of microorganisms against the two extracts was determined using the disk diffusion method. Preliminary phytochemical screening showed the presence of saponins and terpenoids in both extracts. Alkaloids, tannins, and flavonoids were found only in methanol extract, whereas coumarins and steroids were found only in chloroform extract. The chloroform and methanol extracts were analyzed using gas chromatography-mass spectroscopy (GC-MS), which showed the presence of many biologically important compounds. The major compounds identified in chloroform extract were phenol,3-pentadecyl (36.21%), benzene,1-(1,1-dimethylpropoxy)-4-methyl (14.53%), and isobutyl-3-methylpyrazine (9.17%). In comparison, the major compounds identified in methanol extract were 1,2,3-benzenetriol (24.4%), benzoic acid,3-hydroxy (22.69%), and quinic acid (20.41%). Methanol extract exhibited significant activity against *S. aureus* (22 mm), *B. subtilis* (19 mm), *P. aeruginosa* (17 mm), *E. coli* (15 mm), and *C. albicans* (8 mm). Whereas chloroform extract exhibited moderate inhibitory activity only against *C. albicans* (10 mm). The findings of this study indicated that the fruit of *P. lentiscus*possesses various secondary metabolites having the potential for developing antibacterial agents.

***Keywords*:** *Pistacia lentiscus;* antibacterial activity; antifungal activity; GC-MS analysis.

Introduction

Public health problems have increased rapidly due to the increasing rate of infectious diseases, and the interest in using medicinal plants to fight against the growth of pathogenic strains such as fungi and bacteria is increasing. [1] Due to many artificial antibiotics being more expensive and showing serious side effects, people's attention is turned to herbal medicine. [2, 3] According to the World Health Organization (WHO), nearly 80% of the population in developing countries relies mainly on traditional medicine for their healthcareneeds, of which a major portion involves the use of plant extracts. [4] Worldwide, many studies are running to verify the therapeutic effects and safety of some medicinal plants, and it has been referred to their secondary metabolites as having different health-promoting bioactivities. [5, 6]

P. lentiscus is a deciduous fruit of the Anacardiaceae family. It is widely distributed in Mediterranean countries like Libya, Morocco, Tunisia, and Algeria. [7] The Green Mountain region, especially the outskirts of Al-Bayda city (northern east of Libya), is rich in wild-growing trees of *P. lentiscus,*whosefruits are usually eaten by residents after roasting them on fire.

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Different parts of this plant (e.g., fruits, leaves, and roots) have been used widely to treat various diseases and ailments. The most marked biological effects are its antibacterial, [8, 9] antioxidant, [10, 11] anti-inflammatory [12], and anticancer activities. [13, 14] Moreover, essential oils from different parts (e.g., fruits, leaves, roots) of *P. lentiscus* inhibited the growth of many and various bacteria and fungi strains as reported in a review article elsewhere. [15]

Besides, its use in traditional medicine, *P. lentiscus* has been employed in the food, spices, cosmetic, and pharmaceutical industries. [16-18] Its extracts contain various medicinally necessary compounds such as polyphenols, oleanane, alpha-tocopherol, and euphane. [19] Essential oil obtained from leaves turned out to contain *γ*-cadinene (6.48%), germacrene (12.05%), and *β*-caryophyllene (31.38%). [20] In the literature, there is some work reported by authors on different parts of *P. lentiscus* summarized in a recent review article reported elsewhere. [21] *P. lentiscus* has received growing attention globally for its chemical composition and medicinal value. However, only limited information is available on *P. lentiscus* grown in Libya. Therefore, the aim of this work was to identify the chemical composition of Libyan plant *P. lentiscus* fruit using GC-MS and determine its antimicrobial activity.

Materials and Methods

Plant material

Fresh fruit of *P. lentiscus* were collected in August 2022 from the Green Mountain Region in the Northern East of Libya. The plant was authenticated and identified by a Botanical specialist; Dr. Hussein Altagori at the Department of Plants, Faculty of Science, Benghazi University, where a herbarium specimen (No 2561) was deposited.

The fruit was washed with water, dried in the shade then grounded into small pieces using an electrical grinder.

Microbial strains
The antibacterial activity of *P. lentiscus* fruit extracts was tested against the following microorganisms: *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853), whereas the antifungal activity was tested against *Candida albicans* (ATCC 7596). All microorganism strains were provided by the Medicinal and Aromatic Plants Institute, National Center for Research, Khartoum, Sudan. To prepare a microbial suspension, the bacterial strains were cultured overnight at 37 °C in nutrient agar, while the fungal organism was cultured for three days at 37 °C using potato dextrose agar. The next day, the growth was harvested, dissolved in normal saline, and calibrated with 0.5 MacFarland solution to prepare for the sensitivity assay.

Preparation of fruits extracts
For qualitative and quantitative comparison, the grounded fruit (20 g) was extracted using a semi-continuous extraction method (Soxhlet) for six hours with two solvents, chloroform and methanol. The organic solvent was evaporated by the rotary evaporator and further dried under open air. Finally, the percentage of each extract was calculated (w/w%) stored in a dark bottle, and kept at 4°C until analysis or evaluated for antibacterial activity as described as follows:

Phytochemical screening
Preliminary phytochemical screening of secondary metabolites such as alkaloids, anthraquinones, coumarins, flavonoids, saponins, steroids, tannins, and terpenes was carried out according to the standard phytochemical methods.[22]

GC-MS analysis
Chloroform and methanol extracts of *P. lentiscus* fruits were analyzed using a Shimadzu GC-MS (Model QP2010-Ultra, Japan) equipped with Rtx-5MS capillary column (30 m × 0.25 mm × 0.25µm). An electron ionization system with an ionization energy of 70 eV was used to detect the chemical constituents. Helium was used as a carrier gas at a flow rate of 1.61 ml/min, and the mass transfer line and injector temperature were set at 250 and 300 °C, respectively. The temperature program was started from 60 °C with a rate of 10 °C/min to 300 °C as the final temperature degree with 10 minute hold time. The sample (1 µl) was injected in the split mode with a split ratio of 120:1, the delay time was 2 min and the total running time was 29 min. [23] Identification of constituents for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST).

Antibacterial activity
The antibacterial activity of fruit extracts was tested using the agar disc diffusion method. [24] The test was conducted in sterile Petri dishes (60 mm diameter) containing 20 ml Muller Hinton agar medium. Briefly, the suspension of the tested microorganism (0.1 ml of 10^7 CFU/ml) was spread on the Muller Hinton agar. A volume of 20 µl of each extract absorbed on sterile paper discs was placed on the previously inoculated media surface. One filter paper disc was placed per Petri dish to avoid a possible additive activity. One filter paper disc was placed per Petri dish in order to avoid a possible additive activity. Every dish was incubated at 37 °C for 24 hours, followed by measuring the zone diameter of the inhibition expressed in mm. All the tests were conducted in triplicate and the data were presented as the mean values with their standard deviation. The measurement scale was as follows: zone < 9 mm had weak inhibitory performance, zone of 10-12 mm had a moderate inhibitory effect, and zone of ≥ 13 mm had strong growth inhibition activity.[24]

Antifungal activity
The antifungal activity of fruit extracts was determined by the disc diffusion method using a Sabouraud dextrose agar medium. The process was maintained as in the above antibacterial method, with only one difference in incubation time which lasts for three days.

Statistical analysis
The results obtained in this study were analyzed using SPSS software (SPSS Statistical Version 22). All values presented are mean values ± standard deviation of triplicates (n = 3), obtained from three separate experiments. The one-way ANOVA and post hoc multiple comparison tests (Scheffe) were performed to examine the differences among the groups. A P value of < 0.05 was considered to be statistically significant.

Results and Discussion

Extraction yields
Yields of the extracts are expressed in percentage and refer to the dry weight of the plant material used (20 g). The yield of the methanol extract was 19.2%, whereas chloroform yield was 16%. For quantitative comparison, the yield obtained by methanol was higher than that extracted by chloroform. The extraction yield was used to indicate the effects of the solvents used. The polarities of the solvents influence the different yields of extract. [25] Several studies have reported variations in the chemical composition of extracts prepared using different solvents. Thus, it is necessary to select the suitable solvent based on the chemical properties of the analytes, sample matrix properties, efficiency, matrix-analyte interaction, and desired properties. [26]

Phytochemical screening
Preliminary phytochemical screening of the extracts showed the presence of various phytochemicals, including alkaloids, coumarins, flavonoids, saponins, tannins, and terpenoids. No anthraquinone was detected in both of the extracts tested (Table 1). Methanol and chloroform extracts both showed the presence of higher amounts of terpenoid compounds. They also showed the presence of saponins by the formation of a 1.5 cm thick foam layer in the test tube. Methanol extract also confirmed the presence of flavonoids. In comparison, chloroform extract confirmed the presence of coumarins. When compared to the chloroform extract, the abundance of alkaloids in the methanol extract was evidenced by the formation of a strong yellow precipitate in the test tube by Hager's test. Phytochemical analysis by Mohammed *et al.* [27] revealed the presence of alkaloids, coumarins, flavonoids, saponins, tannins, and terpenoids in *P. lentiscus* fruit extract, which agrees with our results (Table 1).

GC-MS analysis
Figure 1 shows the spectrum of compounds detected from the GC-MS analysis. The GC-MS analysis indicated the presence of 16 and 17 compounds in the chloroform and methanol extracts, respectively, which included a wide range of phytochemicals, including fatty acids, phenolics, heterocyclic, aromatics, terpenes, hydrocarbons, and phytosterols. The identity, molecular formula (MF), retention time (RT), and area % observed for each compound were expressed in Table 2.

Table 1: Preliminary phytochemical screening of fruit extracts

Phytochemical test	Methanol extract	Chloroform extract
Alkaloids	+	–
Anthraquinones	–	–
Coumarins	–	+
Flavonoids	+	–
Saponins	+	+
Steroids	–	+
Tannins	+	–
Terpenoids	+	+

+: present; ++: moderately present; +++: strongly present; –: absent

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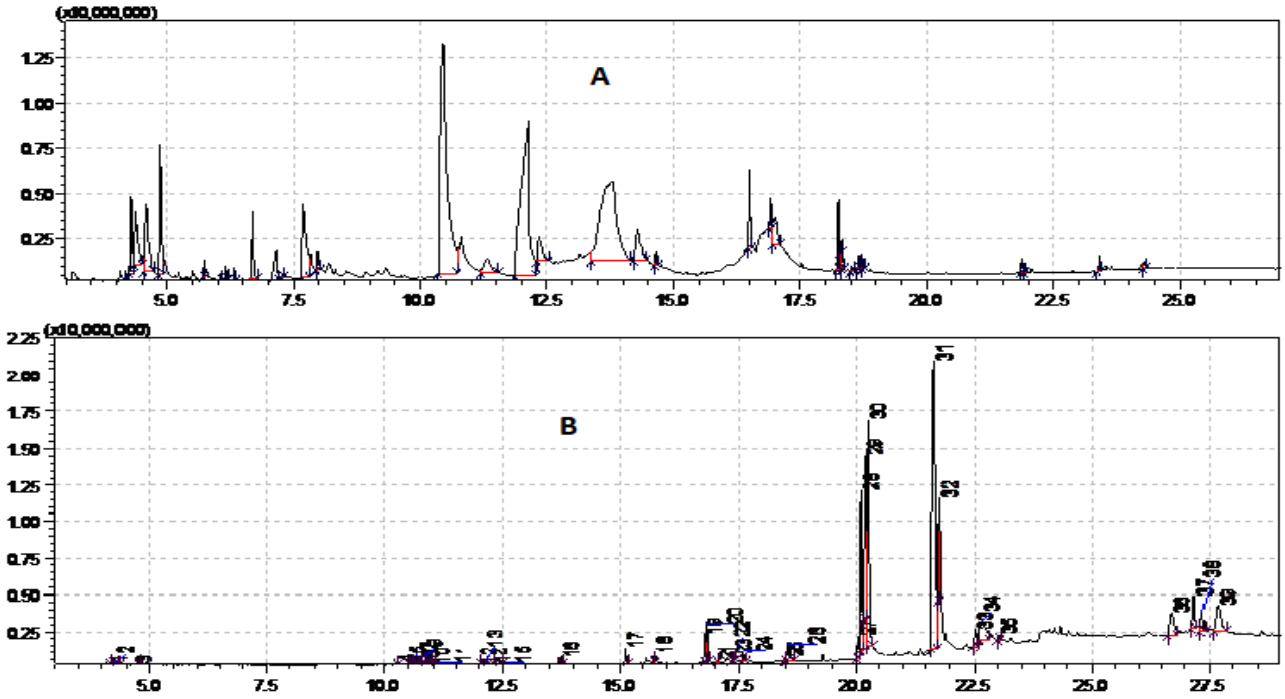

Figure 1: Gas chromatogram of chloroform (A) and methanol (B) extracts of *P. lentiscus* fruit using GC-MS

The major compounds identified in chloroform extract were, phenol,3-pentadecyl (36.21%), benzene,1-(1,1-dimethylpropoxy)-4-methyl (14.53%), isobutyl-3-methyl pyrazine (9.17%), isoamyl-6-methylpyrazine (8.56%), lup-20(29)-en-3-one (5..86%), butyl-3-methylpyrazine (4.59%), indole, 2-pentadecyl-3-phenyl (3.92%) and *α*-sitosterol (3.26%). Whereas the major compounds identified in methanol extract were, 1,2,3-benzenetriol (24.4%), benzoic acid,3-hydroxy (22.69%), quinic acid (20.41%), catechol (4.51%), 2-cyclohexen-1-one (4.18%), and 1,2-cyclohexanedione (3.8%).

The abundance of terpenoids compounds such as neogammacer-22(29)-ene, lup-20(29)-en-3-one, *α*-acorenol, *α*-muurolene, *ß*-copaene, carene, *α*-terpinene, in *P. lentiscus* extract indicate for many health benefits such as antiviral, anticancer, anti-inflammatory, antimicrobial, anti-allergenic, antihyperglycemic, antispasmodic, antiparasitic, and immunomodulatory properties. [28-31] There are remarkable differences in the chemical composition of Libyan *P. lentiscus* fruit with previous studies regarding the same species growing in Tunisia, [32] Algerian, [33] or elsewhere. For example, Libyan *P. lentiscus* contains a high proportion of phenol,3-pentadecyl (36.21%) compared to Tunisian *P. lentiscus* which contains only 14.11% phenol,3-pentadecyl. The composition of certain substances may significantly increase or decrease under specific environments. [34] It is demonstrated that medicinal plants that grow in various environments produce different active substance contents because of their wide distribution in different geological zones. [35] This will result in variations in their internal quantities in the same species from different growing countries, [36] making the quantities assessment of widespread species *P. lentiscus* extremely crucial.

Antimicrobial activity

The results of the antimicrobial activity of methanol and chloroform extracts of the fruits of *P. lentiscus* tested against different pathogenic microorganisms are represented in Table 3. Methanol extract exhibited significant activity against *S. aureus*(22.3 mm), *B. subtilis* (19.1 mm), *P. aeruginosa* (17.3 mm), *E. coli* (15.4 mm), and *C. albicans* (8.0 mm). Although the inhibitory activity showed by the chloroform extract was moderate against the fungus *C. albicans* (10.0) mm), this extract could not inhibit the growth of all tested bacteria.

Generally, *Gram*-negative bacteria are almost less sensitive to the antibacterial agents of plant sources. [37] Our current investigation revealed that fruit methanol extract possessed a significant antibacterial effect and mainly inhibited the proliferation of *Gram*-positive bacteria, especially *S. aureus* (22.3 mm). A possible explanation for this observation may be associated with the significant structural differences between *Gram*-positive and *Gram*-negative bacterial cell walls, as the latter possess an outer membrane and a unique periplasmic space. [38, 39] In addition, tannins and alkaloids which were detected in abundance in methanol extract, may be responsible for the high antibacterial activity of the methanol extract (Table 2). Tannins are well known for their antibacterial activity and astringent property. [40] Whereas, alkaloids are a large and structurally diverse group of compounds with significant antibacterial properties. Recently, the antibacterial activity of alkaloids has been extensively evaluated in biomedical investigations. Research on the antibacterial mechanism of natural alkaloids reveals that they can disrupt the bacterial cell membrane, [41] affect the DNA function, [42] and inhibit protein synthesis. [43]

In a previous study, Bendifallah *et al*. [44] investigated the chemical constituents and antibacterial activity of crude extract of *P. lentiscus* grown in Algeria and found that the crude extract was high in tannins, flavonoids, and saponins. Furthermore, the extract had strong antimicrobial activity against some species. Missoun *et al*. [45] also confirmed that the methanol extract of *P. lentiscus* exhibited a significant zone of inhibition against *S. aureus* and *E. coli*, and the extract contains alkaloids, flavonoids, saponins, glycosides, tannins, steroids, and terpenoids.

Conclusion

The overall result of this study concluded that methanol extract exhibited significant activity against *S. aureus* (22 mm), *B. subtilis* (19 mm), *P. aeruginosa* (17 mm), *E. coli* (15 mm), and *C. albicans* (8 mm). In comparison, chloroform extract exhibited moderate inhibitory activity only against *C. albicans* (10 mm). The phytochemicals profile of the studied methanol and chloroform extracts showed that *P. lentiscus* fruit is a rich source of alkaloids, saponins, terpenoids, tannins, flavonoids, coumarins,and steroids. Furthermore, evaluating the active constituents responsible for the antibacterial activity requires extensive investigation.

Conflict of Interest

The authors declare no conflict of interest.

Table 2: GC-MS analysis of *P. lentiscus* fruits chloroform and methanol extracts

No	Compound name	Area%		RT
		Chloroform	Methanol	
1	2-Pentenal, (E)-	–	2.17	4.28
2	Phenol	–	2.46	4.37
3	1,2-Cyclohexanedione	–	3.8	4.58
4	2-Cyclohexen-1-one	–	4.18	4.86
5	Pyran-4-one, 2,3-dihydro-3,5-dihydrox	–	1.71	6.68
6	Benzoic acid	–	1.5	7.16
7	Catechol	–	4.51	7.68
8	5-Hydroxymethylfurfural	–	0.50	7.96
9	1,2,3-Benzenetriol	–	24.4	10.44
10	Methyl-trans-2,trans-4-hexadienrdioic a	–	1.45	11.33
11	Benzoic acid, 3-hydroxy-	–	22.69	12.13
12	Nonadecene	0.33	–	13.72
13	Quinic acid	–	20.41	13.77
14	Shikimic acid	–	2.4	14.28
15	Hexadecanoic acid	0.83	2.25	15.12
16	Behenic alcohol	0.43	–	15.74
17	Octadecadienoic acid	3.09	2.16	16.76
18	p-cresyl isovalerate	1.29	–	18.55
19	Epoxycyclooctene,5,5-dimethyl	1.16	–	20.04
20	Isobutyl-3-methylpyrazine	9.17	–	20.12
21	Isoamyl-6-methylpyrazine	8.56	–	20.20
22	Benzene,dimethylpropoxy)-4-methyl	14.53	–	20.25
23	Phenol,3-pentadecyl	36.21	0.3	21.68
24	Butyl-3-methylpyrazine	4.59	–	21.79
25	Benzoimidazolyl,trichlorophenylpropanol	1.44	–	22.51
26	Indole, 2-pentadecyl-3-phenyl-	3.92	–	22.65
27	α-sitosterol	3.26	0.17	26.75
28	Neogammacer-22(29)-ene	2.43	–	27.19
29	Lup-20(29)-en-3-one	5.86	–	27.35

—: Compound not detected or detected in minor trace < 0.3%

Table 3: Antibacterial activity of *P. lentiscus* fruits extracts (100 mg/ml) against tested microorganisms strains by disc diffusion method.

Microorganism	Inhibition zone mm		
	Chloroform	Methanol	Positive control
B. subtilis (+)	0.0 [a]	19.1 ± 1.4 [b]	29.7 ± 2.4
S. aureus (+)	0.0 [a]	22.3 ±2.1 [b]	31.8 ± 3.0 [c]
E. coli (–)	0.0 [a]	15.4 ± 1.1 [b]	31.4 ± 2.3 [c]
P. aeruginosa (–)	0.0 [a]	17.3 ± 1.4 [b]	39.3 ± 1.1 [c]
C. albicans	10.0 ± 71	8.0 ± 1.1	26.1 ± 1.2 [c]

Data are expressed as the mean ± standard deviation of three separate experiments; means with the different letters in the same row are significantly different at ($p < 0.05$). Positive control for antibacterial activity ciprofloxacin (30 µg/ml) and for antifungal activity fluconazole (30 µg/ml).

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Khartoum, Sudan for providing the laboratory facilities to perform the antimicrobial activity.

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