Original article

Chemical Profile and *in vitro* Antimicrobial Activity of Leaf Essential Oils of *Pistacia lentiscus* L. Grown in Sudan

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Abstract

Background and Objectives: *Pistacia lentiscus* L. (Family Anacardiaceae) commonly known as mastic is used in Sudan for treatment of cough and preparation of traditional perfumes. This study carried out to determine the antimicrobial activity and chemical profile of leaf essential oil from *P. lentiscus* growing in Sudan.

Methodology: Essential oil was extracted by hydrodistillation and then analyzed by gas chromatography coupled to mass spectrometry (GC–MS). Antibacterial activity was determined against two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria as well as two fungi *Candida albicans* and *Aspergillus niger* by disc diffusion method.

Results: *P. lentiscus* dry leaf provided a pleasant essence pale yellow oil with a yield of 2.7%. The oil was dominated by the presence of sesquiterpenes hydrocarbons (32.19%) and oxygenated sesquiterpenes (30.59%), respectively. The major components were caryophyllene oxide (12.82%) followed by β-caryophyllene (10%), α-pinene (8.23%), spathulenol (5.28%) and ß-eudesmol (4.92%) respectively. Antibacterial activity of the oil ranged between 17-21 mm where the highest activity was observed against *E. coli* (21 mm) and *Bacillus subtilis* (20 mm). The oil showed also good antifungal activity against *Aspergillus niger* and *Candida albicans* with inhibition zone values of 20 and 21 mm respectively.

Conclusion: leaf essential oil from *P. lentiscus* grown in Sudan exert beneficial antibacterial and antifungal activities.

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Introduction

Essential oils are mainly used in perfumes, food and pharmaceutical industry and manufacture of soaps, toiletries, cosmetics and paints. Studies have shown that some essential oils possess a variety of biological properties including antimicrobial, anti-inflammatory, antioxidant, antiseptic, repellent activities, among others (Džamić and

Matejić, 2017). Recently, there is a growing tendency to replace synthetic oils by natural ones in the cosmetic, food and pharmaceutical industries (Keville and Green, 2012). The information on Sudanese aromatic plants and essential oils are scanty and often published in dated manuscripts (Yagi *et al.*, 2016).

Pistacia lentiscus L. (Family: Anacardiaceae), is a widely used spice and have many applications in perfumery, flavoring and pharmaceutical industries (Bozorgi *et al.*, 2013). Studies are mainly undertaken to the resin which is known as mastic. In Sudan, mastic mixed with sesame oil is used for treatment of cough. Women prepared traditional perfumes and natural deodorant from mastic.

P. lentiscus is widely distributed in the Mediterranean basin ecosystems where it grows wild (Trabelsi *et al*., 2012). It is reported that different environmental pressures including herbivory and edaphoclimatic conditions lead to genetic modification that can change the chemical composition of plants of the same species and consequently resulted in the formation of chemotypes (Peixoto *et al*., 2015). Studies of chemical profiles of essential oils in relation to environmental factors might provide information on the specific condition that determines its chemical polymorphism and provide quality criteria for their marketing and contributes to their valorization (Zouari *et al.*, 2012). These chemotypes, in turn, can produce essential oils with different biological activities (Guimarães *et al*., 2008) and consequently may have a unique application as oils, in spices and pharmaceutical industries (Lohani *et al.*, 2015). In Sudan, *P. lentiscus* is cultivated in Sudan for its essential oil for commercial purposes. To our knowledge, there are no reports on the essential oil composition of *P. lentiscus* growing in Sudan. One study carried out by Alhadi *et al.*, (2018) evaluated the antioxidant and antimicrobial activities of ethanolic (80%) extracts from the leaf and stem of *P. lentiscus* and their fractions and screened their secondary metabolites. Thus, the aim of the present study was to determine the chemical profile and evaluate the antimicrobial activity of leaf essential oil from *P. lentiscus* growing in Sudan.

Material and methods

Plant material

Leaves of *P. lentiscus* were collected on January 2017 from the district of Shambat-Khartoum North, Sudan. Botanical identification and authentication were performed and voucher specimen No. 2015/4PL have been deposited in the herbarium of Al-Neelain University.

Preparation of essential oil

Essential oil from the leaf (500 g) was extracted by hydrodistillation using a Clevenger-type apparatus for four hours. The extracted oil was dried over anhydrous sodium sulphate and stored at 4° C, in amber-coloured bottle, before use.

Gas chromatography/mass spectrometry (GC–MS) analysis

Analysis of the chemical composition of the essential oil was performed by gas chromatography coupled to mass spectrometry (Model GC-MS-QP5050A, Shimadzu, Japan) equipped with a Rtx-5MS capillary column (5% diphenyl – 95% dimethylsilicone, 30.00 m × 0.25 mm × 0.25 m). The oven temperature was programmed from 45° C for 1 min and then increased at a rate of 3° C min^{-1} to 300° C and held isothermally for 5 min. Helium was used as the carrier gas (with a flow rate of 1 mL min^{-1}). The detection was performed in the full scan mode, with a mass range of 33–450 *m/z*. Electron impact ionization was employed with collision energy of 70 eV and the mass spectrometer ion source was maintained at 230° C.

The volatile compounds were identified by matching mass spectra with those stored in the mass spectral library of the GC–MS system (MassFinder 4, NIST08 and Wiley Registry™ 9th Edition), retention indices and compared with published data. The relative amounts of individual components were expressed as percent peak areas relative to the total peak area.

Antimicrobial activity Test strains and culture media:

Standard strains of microorganism were used in this study and were obtained from Medicinal and Aromatic Institute of Research, National Research Center, Khartoum. The bacterial species used were the Gram-negative bacteria; *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and the Gram-positive bacteria; *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923). Fungal species were *Candida albicans* (ATCC 7596) and *Aspergillus niger* (ATCC 9763). Bacteria were grown in Mueller Hinton Agar and fungi were grown in Sabouraud Dextrose Agar. The concentration of bacterial suspensions was adjusted to 10^8 cells/mL and that of fungal suspensions to 10^7 cells/ml.

Antibacterial assay: Antibacterial activity of the oil was evaluated by the disc diffusion method (Kil *et al.*, 2009). One mg/ml was prepared by diluting with 5% dimethyl sulfoxide (DMSO). The tested microorganisms were seeded into respective medium by spread plate method. After solidification, filter paper discs with a diameter of 6.0 mm were impregnated with 10 µl of sample followed by drying off. DMSO was used as a negative control, while gentamicin (10 µg/disc) was used as a positive control. Antibacterial discs were dispensed onto the surface of the inoculated agar plates and Petri plates were incubated for 24 hours at 37° C. Experiment was done in triplicate. Diameters of clear zone of inhibition produced around the discs were measured and recorded.

Antifungal assay: Antifungal activity was also evaluated by the disc diffusion method (Mothana and Lindequist, 2005). Paper discs were impregnated with 10 µl of oil at 1 mg/ml followed by drying off. DMSO was used as a negative control, while nystatin (10 µg/disc) was used as a positive control. Antifungal discs were dispensed onto the surface of the inoculated agar plates, after which the plates were incubated at 27° C for 48 hours. Experiment was done in triplicate. After the colonies grew, the zones of inhibition around the discs were measured and recorded.

Results

Essential oil: Steam distillation of *P. lentiscus* dry leaf provided a pleasant essence pale yellow oil with a yield of 2.7%. Analysis of the essential oil resulted in the identification of 63 components comprising 98.32% of the total volatile oil (Table 1). The oil was dominated by the presence of sesquiterpenes hydrocarbons (32.19%) and oxygenated sesquiterpenes (30.59%), respectively. Hydrogenated and oxygenated monoterpenes comprised 13.28% and 5.83% respectively of the oil (Figure 1). The major components were the oxygenated sesquiterpenes caryophyllene oxide (12.82%) followed by the hydrogenated sesquiterpene caryophyllene (10%), hydrogenated monoterpene α-pinene (8.23%), oxygenated sesquiterpenes spathulenol (5.28%) and ß-eudesmol (4.92%), respectively.

Antimicrobial activity

The results obtained from the antimicrobial activity study of the leaf essential oils of *P. lentiscus* are shown in Table (2). Inhibition zones obtained from antibacterial activity of the oil ranged between 17-21 mm where the highest activity was observed against *E. coli* (21 mm) and S. *subtilis* (20 mm). The oil showed also good antifungal activity against *A. niger* and *C. albicans* with inhibition zone values of 20 and 21 mm, respectively.

Discussion

In this study the oil of *P. lentiscus* leaf was dominated by the presence of sesquiterpenes hydrocarbons (32.19%) and oxygenated sesquiterpenes (30.59%), respectively. The chemical composition revealed that the major components were caryophyllene oxide (12.82%), β-caryophyllene (10%), α-pinene (8.23%), spathulenol (5.28%) and ß-eudesmol (4.92%) respectively.

This result was generally different from that observed for *P. lentiscus* grown in other regions. For example; samples collected from 14 populations of *P. lentiscus* leaf from Tunisia showed that monoterpene hydrocarbons (41.9%) and sesquiterpene hydrocarbons (40%) constituted the major components of the oil (Aissi *et al.*, 2016).

Table 1: Chemical profile of *Pistacia lentiscus* leaf essential oils

RI	Compund	Area%	R1	Compound	Area %	RI	Compound	Area %
920	α-Thujene	0.09	996	β-Myrcene	0.29	1020	*p*-Cymene	1.26
948	α-pinene	8.23	1005	Octanal	0.03	1018	D-Limonene	2.62
951	Camphene	0.35	1010	Carene	0.10	1059	Eucalyptol	1.57
957	α-Sabinene	0.15	1042	ß-Cymene	0.03	984	2-Carene	0.08
1104	ß-Linalool	0.34	1440	trans-p-2-Menthen-1-ol	0.06	1197	*p*-Cymen-8-ol	0.34
1104	Nonanal	0.16	1136	Verbenol	0.32	1190	L-alpha-Terpineol	1.91
1146	Cis-Verbenol	0.07	1138	Isoborneol	0.17	1191	(-)-Myrtenol	0.09
1231	Geranyl nitrile	0.08	1137	Terpinene	0.38	1119	Verbenone	0.07
1138	2-Norbornanol	0.05	1191	Butanoic acid, 3-hexenyl ester, (E)	0.11	1206	cis-Carveol	0.09
1067	2-Acetylcyclopentan-one	0.09	1579	Humulene	1.25	1502	Tridecanal	2.05
1138	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	0.15	1490	*d*-Guaiene	0.36	1325	9-Isopropyl-1-methyl-2-methylene-5-oxa tricycle (5.4.0.0(3,8))undecane	0.78
1128	p-Menthane	0.05	1480	Valencene	1.13	1238	3-Methyldiadamantane	1.42
1474	1-Tridecyn-4-ol	0.06	1490	Alpha.-Guaiene	2.87	1593	ß-Eudesmol	4.92
1131	Camphenol, 6-	0.40	1419	Viridiflorene	4.50	1656	1-Tetradecanol	1.65
1322	1-Cyclohexanone	0.32	1435	γ-Muurolene	0.75	2251	Etiocholanolone	0.43
992	1,5,5-Trimethyl-6-methylene-cyclohexene	0.13	1386	Aromandendrene	3.31	1601	Tetradecanal	6.67
1403	(-)-Aristolene	0.70	1523	β-Guaiene	2.12	1754	2-pentadecanone, 6,10, 14-trimethyl	0.62
1490	Lavandulol	0.26	1522	Elemol	0.60	1902	Farnesyl acetone	0.39
1494	β-Caryophyllene	10.00	1523	7-epi-cis-sesquisabinene hydrate	2.44	1869	Pentadecanoic acid	1.25
1430	α-Bergamotene	1.01	1677	Caryophyllene alcohol	1.11	2046	Geranyl linalool	0.45
1386	Alloaromadendrene	4.19	1536	Spathulenol	5.28	2045	Phytol	2.13
1754	Nerolidyl acetate	0.59	1507	Caryophyllene oxide	12.82			
							Total Oil yield	98.32 2.7

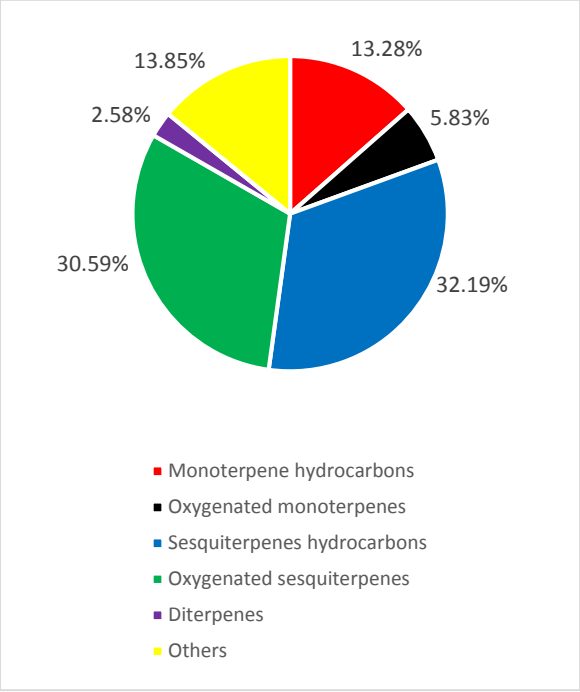

Figure 1: Composition of chemical compound families identified in essential oil.

Those from Morocco were mainly dominated by monoterpene hydrocarbons (Derwich *et al.*, 2010). Furthermore, oil from *P. lentiscus* leaf grown in Tunisia showed that α-pinene (9.9%), limonene (8.5%) and α-terpinol (5.1%) were the major components (Aissi *et al.*, 2016) while in another study α-pinene (17%), γ-terpinene (9%) and terpinen-4-ol (12%) were characterized as the main constituents (Ben Douissa *et al.*, 2005). The oil from *P. lentiscus* leaf grown in Morocco was dominated by α-pinene (24.25%) and β-terpinol (12.58%) (Aissi *et al.*, 2016). Studies on *P. lentiscus* essential oil from Turkey showed that terpinene-4-ol (33.7%), α-terpineol (8.1%), β-caryophyllene (3.2%), and spathulenol (4.6%) were the major constituents while in another study (Kıvçak *et al.*, 2004) terpinene-4-ol (29.2%), β-caryophyllene (29.2%), and *p*- cymene (7.1%) were reported. Congiu et al., (2002) found the main components of the essential oil of the leaf were β-

Table 2: Antimicrobial activity of *Pistacia lentiscus* leaf essential oils.

Microorganism	Inhibition zone (mm)	Gentamicin*	Nystatin*
Bacillus subtilis	17 ± 0.31	16 ± 0.01	-
Staphylococcus aureus	20 ± 0.12	19 ± 0.01	-
Escherichia coli	21 ± 0.37	17 ± 0.01	-
Pseudomonas aeruginosa	18 ± 0.07	16 ± 0.01	-
Aspergillus niger	20 ± 0.33	-	24 ± 0.01
Candida albicans	21 ± 0.18	-	20 ± 0.01

pinene (18.71%), β-phellandrene (12.83%) and β-caryophyllene (13.22%). Most of these compounds were identified in the present study but with lower quantities. Furthermore, although terpinene-4-ol and α-terpineol present in considerable amount in oils from *P. lentiscus* in other regions, it was not detected in this study. Thus, it is clear that oils obtained from *P. lentiscus* growing in Sudan showed quantitative and qualitative differences. This might be attributed to geographical and environmental factors as well as time of harvest and age of the plant which were known as key factors influencing the chemical composition, quality and quantity of the plant essential oil and could probably contribute to create unique chemotypes (El-Zaeddi *et al.*, 2016).

Antimicrobial activity results showed that the essential oil of the leaf displayed good antimicrobial activity. The highest inhibition zones were observed against *E. coli* (21 mm) and *S. subtilis* (20 mm). These values were higher than those obtained from positive control Gentamicin at concentration 10 µg/disc. Also, the oil showed pronounced antifungal activity against *A. niger* (20 mm) and *C. albicans* (21 mm) with inhibition zone values lower for *A. niger* (24 mm) and comparable for *C. albicans* (20 mm) to those observed from the antibiotic Nystatin at concentration 10 µg/disc. *Pistacia* spp, are known to possess significant antibacterial and

antifungal activities (Bozorgi *et al*., 2013). Koutsoudaki *et al*., (2005) suggested that combination of several components including α-pinene, verbenone, α-terpineol, linalool, and carvacrol are major compounds responsible for the antibacterial activity in the gum oil of *P. lentiscus*. Moreover, the antimicrobial properties of essential oils containing a significant sesquiterpene fraction were summarized by de Melo *et al*., (2005). Nascimento *et al.*, (2007) and Santos *et al*. (2008) reported that essential oil rich in caryophyllene oxide and caryophyllene demonstrated high antimicrobial activity. Thus, it could be suggested that the high antimicrobial activity of leaf essential oil of *P. lentiscus* growing in Sudan could be attributed to these two compounds as well as other terpenes as some studies found that whole essential oils possess greater antimicrobial activity and suggested that minor components might be critical to such activity, due to synergistic (or other) effects (Santos *et al*., 2008 and Ramos *et al*., 2013).

Conclusions

*It is worth mentioning that this is the first study for evaluation of chemical profile and antimicrobial activity of leaf essential oil from *P. lentiscus* growing in Sudan.

*Results showed that the oil was readily distinguished from the other *Pistacia* oils where it was dominated by sesquiterpenes contrary to reported studies on *P. lentiscus* growing in Mediterranean region where it was either dominated by monoterpenes or the percentages of monoterpenes and sesquiterpenes were comparable. Caryophyllene oxide and β-caryophyllene were the major components.

*Variability in the qualitative and quantitative composition of *P. lentiscus* oil was most probably associated with the geographical regions in addition to genetic and environmental factors.

*The potential of leaf essential oil to possess beneficial antibacterial and antifungal activities and eventually, it could contribute to solve certain health problems. *Further studies are needed to evaluate other biological properties and to investigate in depth its mode of action.

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