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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF TRUNK EXUDATES OF *Pistacia atlantica* var. *mutica*

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The genus *Pistacia* L. consists of 11 species, most of which are known to produce oleoresin. There are three kinds of pistachio in Iran, including *P. vera*, *P. atlantica*, and *P. khinjuk*. Four subspecies or varieties have been identified for *P. atlantica*: *cabulica*, *kurdica*, *mutica*, and *atlantica* [1]. *P. atlantica* var. *mutica* is native to a number of temperate countries in Asia. In Iran, this plant grows in the central, western, and eastern regions. The oleoresin of *P. atlantica* var. *mutica*, known as "Turk terebinth gum," is used to make chewing gum in Iran. Mastic gum has been used in traditional medicine for various gastrointestinal disorders like gastralgia, dyspepsia, and peptic ulcer. Mastic gum has been reported to possess considerable *in vitro* antibacterial and antifungal activity. The total mastic extract without polymer might be effective in reducing *Helicobacter pylori* colonization as well as in the treatment of cutaneous leishmaniasis. This plant has also been used traditionally as an antiseptic and as a mouth freshener constituent [2]. The essential oil of *P. atlantica* var. *mutica* has also potential application as an antimicrobial agent in edible films [2, 3]. The objectives of this study were to report the GC-MS analysis as well as the antibacterial activity of the essential oil of the trunk exudate from *Pistacia atlantica* var. *mutica* in order to evaluate its potential application as a natural food preservative.

GC-MS analysis has led to the identification of 21 components, listed in Table 1, representing 98.77% of total compounds. The major constituents of the essential oil of mastic gum were α-pinene (54.97%), myrcene (11.44%), and limonene (10.08%). A high content of α-pinene was also found in the essential oil extracted from the gum of *P. atlantica* var. *mutica* [2], *P. atlantica* var. *kurdica* [4], *P. lentiscus* [5, 6], *P. khinjuk* [7], *P. vera* [8, 9], and *P. terebinthus* [10].

A comparison between the results of Delazar [2] and this work shows that α-pinene is the main constituent respectively of both essential oils (70.00 and 54.97%). On the other hand, no such similarity was found for the other components. The next two main components reported by Delazar are citral (5.72%) and myrtenol (5.31%), whereas our study showed that myrcene and limonene are ranked as the next two main components (11.44 and 10.08%, respectively). Such variations are most likely related to the geographical origin, harvesting time, growing conditions, as well as extraction method [9].

Disc diffusion is one of the most common assays used in the evaluation of antibacterial activity of essential oils [11]. The results of antimicrobial activity of the essential oil and positive controls are presented in Table 2. The essential oil prevented bacterial growth of all studied bacteria except *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is considered one of the most resistant bacteria against antimicrobial compounds [12]. Antimicrobial activity analyzed by the disc diffusion method showed that the oil resin of *P. atlantica* var. *mutica* was most active against *Bacillus cereus*, followed by *Staphylococcus aureus* and *Escherichia coli* O157 H7. These bacteria are the most common bacteria causing food-borne diseases.

With increasing essential oil concentration from 10 to 20 μL per disc, antimicrobial activity was also increased. As can be seen, a concentration of 10 and 20 μL of the essential oil per disc gives an inhibition zone greater than 7 mm, which is considered as a reasonable limiting inhibition zone for an antibiotic [5]. In the case of *Staphylococcus aureus*, the clear zones of essential oil were smaller in comparison with those of ampicillin and streptomycin. However, the results of clear zones for *Bacillus cereus* and *Escherichia coli* were comparable to positive controls. Finally, gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) were more susceptible to the essential oil as compared to gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

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TABLE 1. Chemical Composition of Essential Oil of the Trunk Exudates of *Pistacia atlantica* var. *mutica*

Compound	KI	%	Compound	KI	%
α-Thujene	932	1.4	*Z*-β-Ocimene	1040	1.0
α-Pinene	943	55.0	*E*-β-Ocimene	1052	0.6
Camphene	955	0.7	*allo*-Ocimene	1132	0.1
Sabinene	978	1.9	*trans*-Verbenol	1150	0.8
β-Pinene	982	1.8	Camphor	1152	1.5
Myrcene	995	11.4	*cis*-Pinocamphone	1177	0.3
α-Phelandrene	1008	1.5	*neo*-Verbanol	1186	0.3
α-Terpinene	1015	0.6	α-Copaene	1379	0.7
ρ-Cymene	1029	3.2	*E*-Caryophyllene	1422	2.6
Limonene	1032	10.1	Viridiflorol	1598	0.6
1,8-Cineole	1034	3.1			

TABLE 2. The MIC Value of Essential Oil of the Trunk Exudates of *Pistacia atlantica* var. *mutica*

Bacteria	Pure essence		MIC (ppm)	Positive control	
	10 µL/disk	20 µL/disk		ampicillin	streptomycin
Bacillus cereus ATCC 10876	11.8	14.1	1000	not visible	15.3
Staphylococcus aureus subsp. Aureus ATCC 25923	11.0	13.0	4000	23.8	20.8
Escherichia coli O157 H7	8.4	11.4	> 8000	8.5	14.3
Pseudomonas aeruginosa ATCC 27853	Not visible	Not visible	> 8000	Not visible	10.3

The radius of the zone of inhibition was measured in mm.

This is in agreement with other reports claiming that plant extracts are more active against gram-positive than gram-negative bacteria [11]. The inhibition activity of the oil can be attributed to the high content of α-pinene. It has been reported that α-pinene possessed antimicrobial activity [4, 8, 13].

Raw Material and Essential Oil Extraction. The resin of *P. atlantica* var. *mutica* (mastic gum) was obtained from the City of Marvdasht in Fars Province, Iran. Mastic gum was kept in the refrigerator (4°C) until the day of experiment. The essential oil was extracted from the resin by hydrodistillation using a Clevenger apparatus for 3 h to give a pale yellow oil. The essential oil was stored in the dark at 4°C in an airtight container [2].

Identification of Oil Components. The components of volatile oil from the resin of *P. atlantica* var. *mutica* were identified using gas chromatography (GC) and gas chromatography-mass spectrometric (GC-MS) apparatus. The gas chromatograph (GC) was a Shimadzu GC-17 equipped with an FID detector and a fused-silica column (HP-5MS, 30 m × 0.25 mm i.d., film thickness 0.25 µm). The operating conditions were: oven temperature 60–210°C raised at the rate of 3°C/min then 210–240°C at the rate of 20°C/min; injector temperature 280°C, split ratio 1:10, carrier gas He; detector temperature 280°C.

The GC-MS apparatus was a Varian GC-MS spectrometer consisting of a Varian star 3400 gas chromatograph equipped with a fused-silica column (HP-5, 30 m × 0.25 mm i.d, film thickness 0.25 µm; J&W Scientific Inc.), interfaced with a mass spectrometric detector (Varian Saturn 3). The operating conditions were: oven temperature 60–280°C raised at the rate of 3°C/min; injector temperature 280°C; injector mode: split injection; carrier gas He; flow rate 0.5 mL/min; mass spectra: electronic impact (EI), ionization potential 70 eV, ion source temperature 250°C, ionization current 1000 µA, resolution 1000, and mass range 40–300 amu.

The oil components were identified from their retention indices (RI) obtained with reference to the *n*-alkane series (Sigma, UK) on an HP-5 column; mass spectra were obtained by comparison with those of authentic samples from mass spectra and fragmentation patterns reported in the literature, as well as by computer matching with the MS-data bank (Saturn version 4). Quantification of the relative amounts of the individual component was performed according to the area percentage method without consideration of a calibration factor.

Bacterial Strains. The gram-positive bacteria (*Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC10876) and gram-negative bacteria (*Escherichia coli* O157 H7 and *Pseudomonas aeruginosa* ATCC 27853) were obtained from the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Determination of Antibacterial Activity. Agar Disc Diffusion Method. The bacteria were first incubated at 37°C for 18 h in nutrient agar slant. The microbial strains were diluted to an optical density of 0.5 McFarland standard at A_{530} with sterile Ringer solution. Then 1 mL of suspension was transferred to a sterile tube with 9 mL Ringer solution to obtain 1.5×10^7 cfu/mL microbial suspension. Mueller-Hinton agar was then poured into the Petri dishes. After solidification, 0.1 mL of inoculum was spread uniformly and dried for 5 min. Subsequently, the sterilized blank paper disks of 6 mm diameter were placed onto the agar plates, which had previously been inoculated with the above organisms. Ten to 20 μL of essential oil was added to each disk. In addition, ampicillin (10 μg per disk) and streptomycin (10 μg per disk) (Padtan-Teb, Iran) were used as positive controls. Afterwards, the plates combined with the disks were kept at 4°C for 2 h, followed by incubation at 37°C for 24 h. After 24 h, the inhibition zones appearing around the disks were measured and recorded in millimeters [9].

Determination of Minimal Inhibitory Concentration (MIC). Bacterial strains were cultured overnight (12–18 h) at 37°C in nutrient agar slant. The microbial strains were diluted to an optical density of 0.5 McFarland standard at A_{530} with sterile Ringer solution. Dilution was made until a microbial suspension of 1.5×10^6 cfu/mL was obtained. A 0.5 mL portion of the suspension was delivered to each tube containing sterile Mueller-Hinton broth (MHB) supplemented with Tween 80 detergent (final concentration 0.5% (v/v)). Then essential oil of different amounts ranging from 2.5 to 80 μL was added. One growth control (MHB + Tween 80) and one sterility control (MHB + Tween 80 + test oil) were also used. The test tubes were incubated under normal atmospheric conditions at 37°C. After 24 h incubation, serial dilution in Ringer solution was made, and Petri dishes containing Mueller-Hinton agar were inoculated and incubated in 37°C for 24 h. The MIC was expressed as the lowest concentration of the essential oil that inhibited visible growth [9].

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