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Original article

Chemical Composition and Biological Effects of *Pistacia atlantica* Desf. Oleoresin Essential Oil

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Abstract

Background and objectives: The oleoresin of *Pistacia atlantica* Desf. (known as "Baneh" in Iran) has been frequently used in traditional medicine for its medicinal properties. Herein, *P. atlantica* essential oil was investigated for its antimicrobial and α-glucosidase inhibitory activities since α-pinene which has been identified as the most abundant component in *Pistacia* genus oil, has demonstrated antimicrobial and anti-α-glucosidase properties. **Methods**: Fresh oleoresin was collected from Javanroud, Kermanshah, Iran and the essential oil was obtained by Clevenger-type apparatus. The chemical composition of essential oil was identified with GC/MS analysis and compared with those reported from various regions. The antimicrobial activity was evaluated against various strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Saccharomyces cerevisiae*, and *Lactobacilli* spp.) through MIC method. Also, its anti-α-glucosidase property and antioxidant activity by DPPH assay were investigated. **Results**: GC/MS analysis of the essential oil confirmed the presence of nineteen compounds and among them, α-pinene (64.8%) was identified as the major constituent. Also, β-pinene (5.7%) and *cis*-limonene oxide (4.5%) were relatively abundant. Our results revealed antimicrobial properties of the "Baneh" essential oil against various bacterial and fungal strains. Moreover, it demonstrated inhibitory activity toward α-glucosidase with IC_{50} value of 41.5 ± 2.5 mg/mL compared with acarbose (IC_{50}=0.5±0.2 mg/mL). DPPH free-radical scavenging activity assay showed antioxidant activity with IC_{50} value of 155.2 ± 1.4 mg/mL compared with quercetin (IC_{50}=250.0±0.0 µg/mL). **Conclusion**: *Pistacia atlantica* oleoresin essential oil depicted satisfactory antimicrobial activity against both Gram-positive and Gram-negative strains. However, it demonstrated low antioxidant and α-glucosidase inhibitory effects.

Keywords: biological activity; chemical analysis; essential oil; oleoresin; *Pistacia atlantica*

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Introduction

The resinoferous wild pistachio tree belongs to *Pistacia* genus which comprises eleven deciduous species and is mainly distributed in the Mediterranean and Middle Eastern areas. *Pistacia atlantica* Desf., known as "Baneh" in Iran, has been traditionally used by indigenous people in the west of the Zagros Mountains (mountain range in western Iran) as a rich source of nutritional and medicinal constituents [1]. It has been widely utilized as an antiseptic, appetizer, phlegm purgative, astringent, laxative, and carminative drug [2]. However, biological properties of the plant have been considered in the recent studies, e. g. *P. atlantica* leaves extracts have depicted remarkable antidiabetic activity under in vitro and in *vivo* [3,4]. Repeated oral administration of the hexane extract of *P. atlantica* seeds has confirmed beneficial hypoglycemic effects on streptozotocine-induced diabetic mice after a two-week treatment [5].

Essential oils are secondary metabolites of the aromatic plants which have demonstrated antioxidant, antibacterial, antiviral, antifungal, and insecticidal activity [6,7]. It has been reported that *P. atlantica* oleoresin essential oil possesses various biological activities such as antibacterial, anti-inflammatory, antifungal, antioxidant and antidiabetic effects [2,8]. Oral administration of *P. atlantica* oleoresin essential oil has been reported to be safe up to higher doses (2000 mg/kg) [8]. Therefore, it is deemed to be safe to use in the food industry [9]. Various studies have also indicated the antibacterial activity of *P. atlantica* oleoresin essential oil against several foodborne pathogens, including *Staphylococcus aureus*, *Salmonella enteritidis*, *Bacillus cereus*, *Escherichia coli* and *Helicobacter pylori* [10,11].

The composition of essential oils is remarkably associated with geographical position, climate conditions, and the method of extraction [12,13]. Different studies revealed that *P. atlantica* oleoresin essential oil contained high amounts of monoterpenes such as α-pinene (25-78%) and β-pinene (3-16%). However, oxygenated monoterpenes and sesquiterpenes were found to be low [14]. In this study, the essential oil of freshly collected *P. atlantica* oleoresin was obtained and the chemical composition was determined with GC/MS analysis. Its antimicrobial activity was evaluated against different bacterial and fungal strains and compared with those results reported previously for the same species from various regions. It should be noted that evaluation of the corresponding essential oil against *Lactobacilli* spp. is reported for the first time. Also, *P. atlantica* oleoresin essential oil was evaluated for its α-glucosidase inhibitory activity which was not considered for the same species from different regions.

Materials and Methods

Ethical considerations

The Ethics Committee of Tehran University of Medical Sciences approved this research by the code IR.TUMS.TIPS.REC.1397.111.

Chemicals

All chemicals including *p*-nitrophenyl glucopyranoside (*p*-NPG), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), quercetin, potassium dihydrogen phosphate, and dipotassium hydrogen phosphate as well as *Saccharomyces cerevisiae* α-glucosidase (EC 3.2.1.20, 20 U/mg) were purchased from Sigma-Aldrich.

Plant material

Pistacia atlantica oleoresin was harvested from Javanroud, Kermanshah Province, Iran (34°48′24″N 46°29′19″E) in July 2018. It was identified and deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Science, Tehran, Iran with the voucher specimen of PMP-890. The collected oleoresin was kept at 4 °C.

Bacterial strains

Antimicrobial activity of the essential oil was assessed against Gram-negative bacteria including *Esherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027), Gram-positive bacteria including *Staphylococcus aureus* (ATCC 6538), *Lactobacillus acidophilus* (ATTC 53671), *Lactobacillus casei* (ATTC 27139*), Lactobacillus reuteri* (ATTC 23272), *Lactobacillus rhamnosus* (ATTC 39595), and *Lactobacillus plantarum* (ATTC 14917) as well as yeasts including *Candida albicans* (ATCC 10231) and *Saccharomyces cervisiae* (ATCC 1611). All microbial strains were provided by Iranian Research Organization for Science and Technology (IROST).

Preparation of the essential oil

The volatile fraction was obtained by hydrodistillation of (100 g) oleoresin for 3 h using Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulfate and stored in a sealed glass vial at 4 °C.

GC/MS analysis

Chemical analysis of the sample was achieved on an Agilent 7890A GC instrument with a flame ionization detector (FID) accompanied with MS-5975C MSD (Agilent Technologies, USA). The silica capillary column was DB-5 Agilent (30 × 0.25 mm ID, film thickness: 0.25 μm; CA, USA) and pure helium (99.999%) was applied as the carrier gas at a flow rate of 1 mL/min. Also, injection volume was 1.0 µL with split ratio of 1:5. The initial oven temperature was held 5 min isothermal at 40 °C and increased to 250 °C at a rate of 50 °C/min for 25 min. Likewise, the MS system was set in electron ionization (EI) mode with a quadruple detector at 70 eV ionization energy. The transfer line and ion source temperatures were set at 250 and 230 °C, respectively. To identify the chemical composition of essential oil, the Kovats retention indices of components were calculated using retention times of an *n*-alkane ladder (C_6-C_{14}) that was injected after essential oil and mass spectra were compared with those reported in the literature. Furthermore, the essential oil compositions were determined by comparing the fragmentation patterns of the peaks with libraries mass spectra (Wiley 7n and NISTO5A). The fragmentation patterns of the mass spectra were also compared with those reported in the literature [15].

DPPH free-radical scavenging assay

The experiment was performed according to the literature with some modification [16]. Briefly, 1 mL of different concentrations of essential oil (3000, 1500, 750 µg/mL) was added to 2 mL solution of DPPH in methanol (1 mM). After incubation at 37 °C for 30 min, the absorbance was read at 517 nm. Quercetin was used at the concentrations of 250, 125, 63, and 32 µg/mL as the positive control. Inhibition percent which indicated the antioxidant activity was calculated by the following equation:

$$I\% = [(A_{Blank} - A_{Sample}) / A_{Blank}] \times 100$$

A_{blank} is the absorbance of the control reaction which contains all components without the tested sample and A_{sample} is the absorbance of the tested sample. The concentration that inhibited 50% of DPPH solution (IC_{50}) was calculated by plotting the inhibition percent against sample concentrations. The test was performed in triplicate and the IC_{50} values were reported as means ± SD.

Evaluation of antimicrobial activity

To obtain the minimum inhibitory concentration (MIC), quantitative tests were performed employing microdilution broth method using 96 U-shaped well plates [17]. Different concentrations of the essential oil (1843, 921, 460, 230, 115, 58, 29, 14.5, 7.2 µg/mL) were prepared based on the literature to evaluate the activity against the bacteria including *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Lactobacillus acidophilus* ATTC 53671, *Lactobacillus casei* ATTC 27139, *Lactobacillus reuteri* ATTC 23272, *Lactobacillus plantarum* ATTC 14917, and *Lactobacillus rhamnosus* ATTC 39595 as well as two species of fungi including *Saccharomyces cerevisiae* ATCC 1611 and *Candida albicans* ATCC 10231. Ciprofloxacin (8 µg/mL) and nystatin (30 µg/mL) were used as the standard antibacterial and antimycotic controls, respectively. For this purpose, the fungi and lactobacilli were cultured on Sabouraud dextrose and De Man, Rogosa and Sharpe (MRS) broth, respectively. Mueller-Hinton was also used for *S. aureus*, *E. coli*, and, *P. aeruginosa*. The essential oil samples at the aforementioned concentrations were added to 1×10^6 CFU/mL of each microorganism. Positive control (cultured along with the microorganism without the essential oil) and negative control (cultured without the microorganism) were also examined. After 24 h of incubation at 37 ºC for bacteria and 25 ºC for fungal species, the microdilution plates were investigated for the absence or presence of visible growth in comparison to sample-free control well. Each experiment was repeated three times with similar results. The endpoint of MIC was the lowest concentration of the essential oil at which the test strain did not demonstrate visible growth.

α-Glucosidase inhibition assay

The assay was performed according to previously

reported methods with some modifications [18]. Briefly, a mixture of phosphate buffer saline (135 µL, pH 6.8, 50 mM), solution of α-glucosidase (20 µL), and the essential oil at different final concentrations in range of 1344-10.5 µg/mL (20 µL) was pre-incubated at 37 °C for 10 min in a 96-well plate. Then, *p*-NPG (25 µL, 4 mM) was added to each well and the absorbance change was recorded at 400 nm within 20 min at 37 °C. The percent inhibition of all concentrations, control, and acarbose (positive control) was calculated using the following formula:

$$\% \text{ Inhibition} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

IC_{50} values were calculated from the nonlinear regression.

Results and Discussion

The *P. atlantica* oleoresin essential oil was obtained using a Clevenger-type apparatus by hydrodistillation of freshly collected oleoresin with 25% w/w yield. It was a viscous liquid in light-yellow colour, possessing a pungent odor and the density of 0.84 g/cm^3. The composition of EO was determined using GC/MS analysis (Table 1). Nineteen compounds were identified, which accounted for 99.2% of the total composition. It should be noted that 75.1% of those compounds belonged to the monoterpenes and 24.1% were oxygenated monoterpenes. Among them, α-pinene (Table 1, entry 1) was the main constituent (64.8%). β-Pinene (5.7%), *cis*-limonene oxide (4.5%), *trans*-pinocarveol (3.3%), *trans*-verbenol (2.7%), α-campholenaldehyde (2.6%), and 8-hydroxy-*p*-cymene (2.3%) were also identified [19-34].

Comparing our results with those reported in the literature (Table 2) obtained from the same species in different parts of Iran, it was found that α-pinene is the most abundant compound; however, its quantity is depended on geographical region of the harvested plant. Accordingly, α-pinene was obtained in a wide range of 55.0-97.2 % [35-42]. The amount of α-pinene in *P. atlantica* oleoresin essential oil from different parts of Algeria was reported in the range of 25.4-79.8% [43,44]. It should be noted that chemical composition of the essential oil from the oleoresin grown in Iraq and Morocco demonstrated α-pinene of 57.1 and 42.9%, respectively [45,46].

Table 1. Chemical composition of *Pistacia atlantica* oleoresin essential oil, identified by GC/MS analysis

Entry	Compound	KI^c	KI^{ref}	Percentage
1	α-Pinene	973	965	64.8
2	Camphene	978	968	0.9
3	Sabinene	1002	991	0.9
4	β-Pinene	1020	999	5.7
5	*p*-Cymene	1030	1026	1.3
6	Limonene	1035	1031	1.5
7	Eucalyptol	1039	1039	0.6
8	2,3-Epoxypinane	1109	1103	1.0
9	α-Campholenaldehyde	1121	1122	2.6
10	*cis*-Limonene oxide	1137	1138	4.5
11	*trans*-Pinocarveol	1154	1149	3.3
12	*trans*-Verbenol	1158	1150	2.7
13	Pinocarvone	1174	1170	1.5
14	8-Hydroxy-*p*-cymene	1195	1194	2.3
15	Myrtenol	1207	1208	1.6
16	Verbenone	1221	1221	1.0
17	*trans*-Carveol	1227	1226	1.0
18	Carvone	1259	1257	1.0
19	Bornyl acetate	1293	1293	1.0
Monoterpenes				75.1
Oxygenated monoterpenes				24.1
Sesquiterpenes				-
Total identified				99.2

KI^c: Calculated Kovats Index, KI^{ref}: Reference Kovats Index

Table 2. Chemical constituents (%) of *Pistacia atlantica* oleoresin essential oil from different regions

Geographical region / Components	*Iran	°Iran	Algeria	Iraq	Morocco
α-Pinene	64.8	55.0-97.2	25.4-79.8	57	42.9
β-Pinene	5.7	1.3-6.7	3-16.5	9.8	13.2
Limonene oxide	-	9	-	-	-
Myrcene	-	11.4	-	-	-
1,8-Cineol	0.6	0.6, 3.1	-	-	-
trans-Verbenol	2.7	2.7	-	3.8	-
trans-Pinocarveol	3.3	5.7	-	2.9	3.2
Carveol	-	2.2	-		-
Sabinene	1	1.2-4.5	-		-
p-Cymene	1.3	1.1, 1.2, 1.8	-	1.3	-
p-Cyminene	-	1.2, 1.4	-	-	-
Citral	-	5.7	-	-	-
Myrtenol	1.6	5.3	-	-	2.0
α-Terpinolene	-	1.3, 1.6, 1.8	-	-	-
Camphen	1	1.3, 1.4	-	1	-
Myrtenal	-	1.1, 1.4	-	1.7	-
Bornyl acetate	1	1.5	-	-	-
D-limonene	1.5	1.5, 10.1	5.1	-	2.0
Thujene	-	1.4	-	-	-
E-caryophyllene	-	2.6	-	-	-
Pinocarvone	1.5	-	5.5	-	-
Δ-2-Carene	-	1.1	-	-	-
neo-Isoverbenol	-	1.3, 1.4	-	-	-
cis-Limonene oxide	4.5	1.1-4.2	-	-	-
α-Phelandren	-	1.8	-	-	-
α-Campholenaldehyde	2.6	1.9	-	1.9	-

*Iran: The result of chemical analysis of *Pistacia atlantica* oleoresin essential oil collected from Javanroud; °Iran: The result of chemical analysis of *P. atlantica* oleoresin essential oil collected from other parts of Iran

The *P. atlantica* oleoresin essential oil showed in vitro antioxidant activity (>50%) at high dose of 2000 mg/mL with IC_{50} value of 155.2±1.4 mg/mL compared with quercetin as the positive control (IC_{50}=250.0±0.0 µg/mL). This result was in accordance with identified compounds (Table 1), indicating a high level of monoterpens.

The calculated minimum inhibitory concentration (MIC) values against examined strains were reported in Table 3. According to our results, the essential oil demonstrated antimicrobial activity against most microorganisms tested with MIC lower than 1 mg/mL. Among them, the essential oil showed the MIC value of 12.6 µg/mL against *L. acidophilus*. Also, it inhibited growth of *P. aeruginosa* and *C. albicans* with MIC value of 37.8 µg/mL. Antimicrobial activity of the essential oil against *S. aureus, E. coli, L. reuteri, L. plantarum*, and *L. rhamnosus* was found to be 115.2 µg/mL. However, it was not efficiently active toward *S. ceriviseae* and *L. casei* (MIC > 1 mg/mL).

Table 3. MIC values (µg/mL) of *Pistacia atlantica* oleoresin essential oil, ciprofloxacin, and nystatin against microbial strains

	MIC (µg/mL)		
Bacteria/fingi	**EO***	**Ciprofloxacin**	**Nystatin**
Staphylococcus aureus	115.2	0.5	-
Escherchia coli	115.2	1.0	-
Pseudomonae aeruginosa	37.8	2.0	-
Saccharomyces ceriviseae	1034.5	-	30.0
Candida albicans	37.8	-	30.0
Lactobacillus acidophilus	12.6	1.0	-
Lactobacillus casei	1034.5	1.0	-
Lactobacillus reuteri	115.2	1.0	-
Lactobacillus plantarum	115.2	1.0	-
Lactobacillus rhamnosus	115.2	1.0	-

EO: essentialoil

Pistacia atlantica oleoresins grown in different geographical regions have demonstrated antibacterial and antifungal activity [10,47,48]. In this regard, high activity against Gram-positive bacteria has been significant. In our study, the more considerable activity of *P. atlantica* oleoresin essential oil was obtained against Gram-negative bacteria which are more resistant than Gram-positive type and can be considered for the respiratory infections. Also, good activity of the essential oil against *L. acidophilus* probably makes it applicable for oral health care. Antimicrobial activity of the essential oil seems to be associated with the presence of α-pinene. Based on the literature, this component has shown good activity against pathogenic yeasts or molds [49-52].

The antimicrobial activity of the essential oil can be explained by high hydrophobicity, which is directly correlated to log *P* (partitioning behavior of the lipophilic compounds in octanol/water) that targets the microbial cytoplasmic membrane [53]. Indeed, lipophilic compounds particularly those with log *P* higher than 3 possess high affinity for cell membranes, and their accumulation induces changes in membrane physico-chemical properties. In this respect, α-pinene, *p*-cymene, and β-pinene (monoterpens) as the most abundant constituents of the EO, are lipophilic compounds and have log *P* values in the range of 3.1-4.1. Also, oxygenated monoterpenes which accounted for 24.1% of the essential oil could play a more or less important role in the antimicrobial activity. The presence of oxygenated terpenes in trace amount can also increase water solubility and the hydrogen bonding capacity, which are the main factors influencing MIC values of terpenoids against *P. aeruginosa* and *S. aureus* [54,55].

α-Glucosidase inhibitory activity of *P. atlantica* oleoresin essential oil as well as the major components (α-pinene and β-pinene), were evaluated and IC_{50} values were obtained as 41.5±2.5, 0.2±3.2, and 0.49±1.4 mg/mL, respectively, compared with acarbose as the positive control (IC_{50}=0.50±0.2 mg/mL). The inhibitory activity may be associated with the presence of monoterpenes such as α-pinene and β-pinene which showed more potent activity than acarbose. However, the synergistic effect led to the reduction of activity.

In the case of inhibitory activity against α-glucosidase, which plays a key role in oligosaccharide hydrolysis, essential oils have been found as potent sources of natural inhibitors [56-59]. Some reports have also revealed that terpenes, as the main components of various plants, are capable of exerting blood glucose lowering effects and monoterpenes such as α-pinene, β-pinene, myrcene, 1,8-cineole, and sabinene have been reported as the potent inhibitors of α-amylase and α-glucosidase [60]. The essential oil of *Hertia cheirifolia* flowers indicated significant α-glucosidase inhibitory effect (IC_{50}=0.24±0.01, acarbose=0.28±0.01 mg/mL). Its composition was characterized by the predominance of α-pinene accounted for 70.4% of the oil [61]. However, in this study the presence of α-pinene as the major compound induced no satisfactory anti-α-glucosidase

activity. It seems that the inhibitory activity was affected by the whole components.

Conclusion

In this study, chemical composition and biological activities of the *P. atlantica* oleoresin essential oil which was collected from Javanroud, Kermanshah, Iran was investigated. According to the GC/MS analysis, α-pinene was detected as the most abundant component in the essential oil (64.8%). It showed broad-spectrum antibacterial activity against different gram-positive and gram-negative bacteria as well as two fungal strains, even against the multi-resistant bacteria, *P. aeruginosa*. It seems that this oil can be considered as a natural and potent antimicrobial agent. However, *P. atlantica* oleoresin essential oil depicted a weak α-glucosidase inhibitory activity in spite of the fact that its major component displayed potent activity.

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Author contributions

Azam Elyasi performed the experiments and prepared the manuscript; Mina Saeedi participated in the characterization of components and preparation of the manuscript; Mahnaz Khanavi designed and supervised all project steps; Somayeh Mojtabavi contributed to the evaluation of biological activity; Farzad Kobarfard conducted GC/MS analysis; Mohammad Ali Faramarzi supervised biological assays.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

EO: essential oil; GC-MS: gas chromatography-mass spectrometry; MIC: minimum inhibitory concentration; DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: the half maximal inhibitory concentration; ATCC: American type culture collection; SD: standard deviation; *p*-NPG: *p*-nitrophenyl glucopyranoside