Original Article

In vitro antifungal properties of *Pistacia atlantica* and olive extracts on different fungal species

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Abstract

Background and Purpose: *Pistacia atlantica*, which belongs to the *Anacardiaceae* family, grows in the Zagrossian region of Iran. The aim of this study was to evaluate the antifungal properties of *Pistacia atlantica* and olive leaf extracts against different fungal species.

Materials and Methods: In this study, we assessed the activities of olive leaf extracts and *Pistacia atlantica* leaf and fruit extracts against *Candida* species, including *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei*. In addition, antifungal activities against three filamentous species, i.e., *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigates*, were assessed, using the agar-well diffusion method.

Results: The minimal inhibitory concentrations (MICs) values of fruit and leaf extracts from *Pistacia atlantica* ranged 6.25-12.5 mg ml^{-1} and 6.25-25 mg ml^{-1} against the tested *Candida* and *Aspergillus* species, respectively. The olive leaf extracts showed no activity against *Candida* species or *Aspergillus flavus*, while they exhibited antifungal potency against *Aspergillus niger* and *Aspergillus fumigatus* (MIC: 12.5-25 mg/ml). The MICs of the mixture of selected extracts ranged from 6.25 to 25 mg/ml.

Conclusion: Based on the results, the ethanolic extracts of the selected plants exhibited antifungal potency against the tested fungi and could be used as natural antifungal agents.

Keywords: Antifungal, Fungi, Olive, *Pistacia*

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Introduction

Pistacia atlantica or Bene from the *Anacardiaceae* family is a native fruit in Iran. This plant is the most economically important tree species, which grows in the Zagrossian region of Western Iran and is used in traditional medicine (Figure 1). The oil extracted from *Pistacia atlantica* is known as Bene hull oil [1].

Among 11 *Pistacia* species, only three including *Pistacia lentiscus var. Chia*, *Pistacia vera*, and *Pistacia atlantica* grow in Iran. These plants are of high economical and pharmaceutical importance and are regarded as the most important *Pistacia* species [2]. Some *Pistacia* species have been used in traditional medicine as tonic, aphrodisiac, antiseptic, and antihypertensive agents; moreover, these species have been applied for the treatment of dental, gastrointestinal, hepatic, urinary tract, and respiratory tract disorders.

Based on scientific evidence, various parts of *Pistacia* species exhibit significant pharmacological activities, i.e., antioxidant, antimicrobial, antiviral, anti-tumor, anticholinesterase, anti-inflammatory, antihyperlipidemic, antinociceptive, antidiabetic, antiatherosclerotic, and hepato-protective activities [3]. According to a recent literature review on studies focusing on the effects of *P. atlantica* species on bacteria, the extracts were shown to inhibit *E. coli* growth (MIC: 163 µg/ml). However, the hydroalcoholic extracts of *Pistacia atlantica* did not exert any effects on *H. pylori*. The findings indicated the inhibitory effects of hydroalcoholic extracts on the tested bacteria, with the exception of *H. pylori*. On the other hand, phenol/chloroform extraction could have inhibitory effects on *H. pylori* [4].

The fruit, oil, and leaf extracts of *Olea europaea* L. (commonly known as olive) are regarded as important sources of nutrition, applied in traditional and modern medicine. Olive compounds are an essential part of Mediterranean diets, with polyphenols constituting a major part of these compounds [5].

Investigation of polyphenols in olive leaves is of great significance, owing to their positive effects on health, induced by their antihypertensive, antidiabetic, anticarcinogenic, anti-athero-sclerotic, anti-inflammatory, and antimicrobial activities [6]. Therefore, the main objective of the present study was to investigate the antifungal activity of extracts from *P. atlantica* and olive leaf against *Candida* species and filamentous fungi, such as *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*.

Material and Methods

In this study, *P. atlantica* leaves and fruits were collected from the Zagros Mountains, while olive leaves were gathered from North of Iran. The leaves and fruits of the plants were dried in shade and crushed to fine powder.

Preparation of the extracts

The crude ethanolic extracts were dried at room temperature for alcohol evaporation. The dried extracts were stored in sterile bottles at -20°C for further analysis.

Microorganisms and inoculum preparation

The fungal isolates assessed in this study included *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei* from the oral cavity, *Aspergillus niger* and *Aspergillus flavus* isolated from environmental samples, and *Aspergillus fumigatus* from the clinical fungus ball sample. All the selected microorganisms were obtained from the Department of Medical Mycology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

The stock fungi were subcultured on Sabouraud dextrose agar (SDA, Merck, Germany) and incubated overnight at 37°C. A few subcultured colonies were diluted in sterile normal saline to obtain 10^6 colony forming units (CFU/ml) with a turbidity of 0.5 McFarland [7].

Screening for antimicrobial properties

Aliquots (1000 mg) of dried plant extracts were dissolved in 2 ml of 100% dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA), and the final concentration of each plant extract was adjusted to 500 mg/ml. The serial two-fold dilutions of plant extracts were prepared at a concentration range of 3.12-50 mg/ml.

The minimum inhibitory concentrations (MICs) of ethanolic extracts were screened, using the agar-well diffusion method. The aliquot (0.1 ml) of the prepared inoculum (10^6 CFU/ml) from the tested organism was cultured on SDA medium in Petri dishes and was uniformly dispersed, using a sterile spreader.

Afterwards, the wells (7 mm in diameter) were punched onto the SDA medium, using a sterile borer. Each well was filled with 0.1 ml of serial dilutions of the tested plant extracts [7]. Sterile DMSO was used as the negative control, while amphotericin B (antibiotic discs containing 30 meg.) and clotrimazole (10 µg per disc) were used as the positive controls. The plates were incubated at 37°C for 24 h. Moreover, the inhibition zones around the wells were measured in millimeter.

Results

Table 1 summarizes the antifungal properties of olive leaves and *P. atlantica* leaf and the fruit and leaf extracts by agar-well diffusion method against the selected fungal species. The olive leaf extracts were ineffective against *Candida* spp. and *Aspergillus flavus*, whereas they showed positive inhibitory effects on *Aspergillus niger* and *Aspergillus fumigatus* (MIC range: 12.5-25 mg/ml).

The MICs of *P. atlantica* fruit and leaf extracts ranged 6.25-12.5 mg ml^{-1} and 6.25-25 mg ml^{-1} against the tested *Candida* and *Aspergillus* species, respectively. The MICs of the mixture of *P. atlantica* leaf and fruit extracts and olive leaf extracts were within the range of 0.625-25 mg/ml against the tested microorganisms (Figure 1, Table 1 and 2).

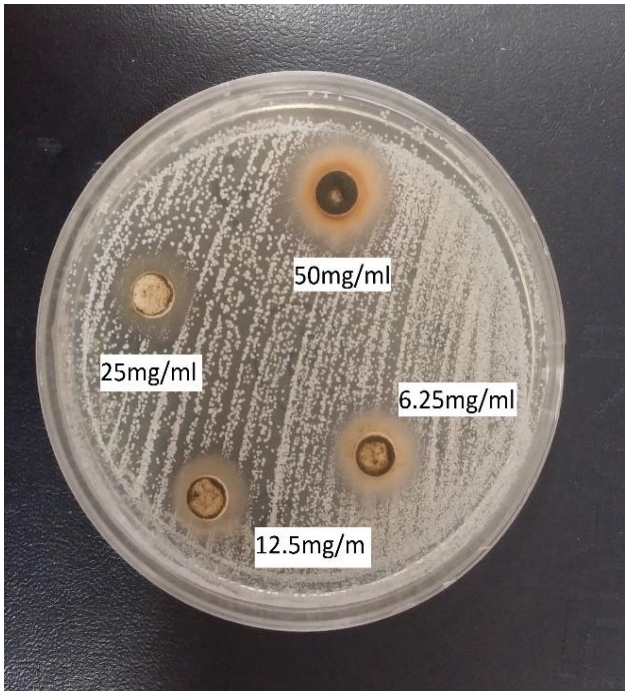

Figure 1. Anti-*Candida* activity assay of the ethanolic fruit extract of *Pistacia atlantica* against *C. albicans* using agar-well diffusion method (dilutions ranging from from 6.25 -50 mg ml^{-1}; MIC = 6.25 mg ml^{-1})

Discussion

In the current study, the ethanolic leaf and fruit extracts of *P. atlantica* and olive leaf extracts exhibited strong antifungal effects. The present findings were in accordance with previous research. In this regard, Hosseini et al. revealed that *P. atlantica* resin extracts could exhibit antibacterial activities against *Streptococcus mutans* and might be useful for oral hygiene during the treatment of dental injuries [8].

In a previous study by Masherghi et al., *Rosmarinus officinalis* extracts showed greater efficiency than *P. atlantica* extracts in inhibiting *E. coli* [9]. Moreover, Torabi and colleagues concluded that the essential oils of *Eucalyptus kingsmillii* and *Eucalyptus salubris* could exert less significant inhibitory effects on *E. coli*, compared to *P. atlantica* extracts [10].

Furthermore, Azizian et al. investigated the effects of *P. atlantica* on bacteria, including *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *H. pylori* and made a comparison with conventional antibiotics. In the mentioned study, MICs of *P. atlantica* extracts were 163 µg/ml, 104.16 µg/ml, and 204.67 µg/ml for *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, respectively, while the extracts showed no significant antifungal

Table 1. Minimum inhibitory concentrations (MICs) (mg/ml)[a] of the ethanolic extracts of *Pistacia atlantica* and olive leaf for *Candida* and *Aspergillus* species

Extracts	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*A. niger*	*A. flavus*	*A. fumigatus*
Pistacia atlantica fruit	12.5	25	6.25	6.25	NI	12.5	6.25
Pistacia atlantica leaf	25	NI	6.25	6.25	25	25	12.5
Olive leaf	NI	NI	NI	NI	25	NI	12.5
[b]Mixure of the three extracts	25	25	6.25	12.5	12.5	25	6.25

[a]Values represent the mean of three replicates; NI: No inhibition; *C.: Candida; A.: Aspergillus,* [b]Leaf and fruit extracts of *Pistacia atlantica* and leaf extract of Olive

Table 2. Inhibition zone (mm)[a] of *Pistacia atlantica* and olive leaf against *Candida* and *Aspergillus* species

Extracts	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*A. niger*	*A. flavus*	*A. fumigatus*
Pistacia atlantica fruit	15	15	20	14	N.I	14	14
Pistacia atlantica leaf	15	N.I	14	13	12	16	12
Olive leaf	N.I	N.I	N.I	N.I	13	N.I	11
[b]Mixture of the three extracts	12	14	13	16	12	13	12
Positive control							
Fluconazole	26	27	17	27	-	-	-
Amphotericin B	-	-	-	-	27	16	21

[a]Values represent the mean of three replicates; NI: No inhibition
Amphotricin B (30 meg/disc) and clotrimazole (10 µg/disc) were used as the positive controls.
[b]Leaf and fruit extracts of *Pistacia atlantica* and olive leaf extract

activity against *H. pylori***.** These findings demonstrated the inhibitory effects of these herbal extracts, although no antifungal activity against *H. pylori* was detected [11].

Paraschos et al. reported the antimicrobial properties of mastic water extract, which is the essential oil obtained from the resin of *Pistacia lentiscus var. Chia*, against *E. coli*, *Staphylococcus aureus*, and *Candida* species. Overall, linalool and a-terpineol constituted the strongest antimicrobial compounds in this plant [11].

In a literature review, the antifungal activity of dichloromethane extracts from olive tree (*Olea Cuspidata* and *Olea Glandulifera* Linn.) against *C. albicans* and *Aspergillus niger* was investigated. The findings revealed maximum antifungal activity of plant extracts against *C. albicans* (inhibition zone of 26 mm) and *Aspergillus niger* with an inhibition zone of 22 mm [13].

Moreover, in the mentioned study, analysis of antifungal activity showed more significant effects against fungi at higher concentrations of dichloromethane extracts. Although individual phenolic compounds in olive extracts could show strong *in vitro* activities, the antioxidant and antimicrobial activities of combined phenolics were similar or more effective than individual phenolic compounds [13].

In the present study, olive leaf extracts showed no significant activity against *Candida* species, while they exhibited antifungal potency against *Aspergillus niger;* this finding was in agreement with previous research [12]. Also, several reports have demonstrated the antimicrobial properties of olive leaf extracts, considering their high phenolic content [13, 14]. In addition, in a previous study, combined phenolics in olive leaf extracts showed similar or better antioxidant and antimicrobial activities than individual phenolic compounds [6].

Faiza et al. revealed strong antimicrobial activity in olive leaf extracts [15]. Also, in a previous study, the antimicrobial properties of *P. lentiscus* and *P. atlantica* extracts against eight bacteria, five molds, and yeasts were revealed. The mentioned study reported that *Klebsiella pneumoneae* and *Escherichia coli* were not sensitive to the ethanolic extracts of *P. lentiscus* and *P. atlantica*. However, *Staphylococcus aureus*, *Salmonella typhimurium*, and *C. albicans* strains were sensitive to these extracts; these findings were in line with the present study.

In the aforementioned study, the ethanolic extracts of *P. lentiscus* showed inhibitory activities against all the tested strains, except for *Rhizopus stolonifer* and *Aspergillus flavus*. It should be noted that the flavonic extracts of *P. lentiscus* exhibited antifungal activities against yeasts and molds, while showing no antibacterial activity [16].

Recent findings are not in agreement with the results of the present study. Paraschos et al. reported the antimicrobial properties of mastic water extract (essential oil of *Pistacia lentiscus var. chia* L. resin) against *E. coli*, *Staphylococcus aureus*, and *Candida* species. Linalool and α-terpineol were reported as the strongest antimicrobial compounds in this plant [11]. These findings were in agreement with previous literature. Also, in the present research, the ethanolic leaf and fruit extracts of *P. atlantica* showed antifungal activities against all the tested strains, except for *C. tropicalis* and *Aspergillus niger.*

So far, several members of the genus *Pistacia* have been chemically investigated. These species are mainly characterized by the presence of flavonoid glycosides and flavonoids, which are hydroxylated phenolic substances. According to *in vitro* studies, phenolic compounds, triterpenoids, and terpenes are effective antimicrobial substances against a wide array of microorganisms; also, it should be noted that two major essential volatile oil constituents, i.e., α-pinene and α-terpineol, possess interesting antibacterial properties [17].

In a previous study, Gokmen et al. screened the antimicrobial activity of *Olea europaea* (olive) leaf extracts against Gram-positive and Gram-negative bacteria. A similarity was detected between the inhibitory zones of olive leaf extracts and commercial antibiotics such as gentamicin against *Salmonella typhimurium*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*.

In the aforementioned study, the MICs of olive leaf extracts against *Listeria monocytogenes*, *E. coli*, *Enterobacter sakazakii*, and

Pseudomonas aeruginosa were ≥ 32 mg/ml. On the other hand, the MIC values against other bacteria (i.e., *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus vulgaris*, *E. coli*, and *Salmonella typhimurium*) were ≥ 16 mg/ml. These findings revealed that olive leaf extracts could be used in medicine, pharmaceutical industries, and food products as natural antimicrobial food additives [18].

Conclusion

As the findings revealed, olive leaf extracts and *P. atlantica* leaf and fruit extracts showed considerable antifungal activities; also, the mixture of the extracts exhibited the same antifungal potency. According to previous research, the strong antifungal activity of olive and *Pistacia* species could be probably attributed to the phenolic and triterpenoid compounds of these plants [6,7,17]. Therefore, these plants could be used as natural antifungal agents to overcome resistance to antibiotics for the treatment of infectious diseases.

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Authors' Contributions

Z.Sh. and M.Z. obtained the specimens and performed all the tests. B.S. wrote, designed, reviewed, and edited the article, and S.YN. prepared and authenticated the medicinal plants.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

Financial disclosure

The authors declare no financial interests related to the materials of the study.

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