

Antifungal Efficacy of *Myrtus communis* Linn

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Background: The ethanolic extract of *Myrtus communis* Linn. leaves was assayed in vitro as a growth inhibitor against opportunistic fungi such as *Candida* and *Aspergillus* species. *Myrtus communis* Linn. (Family, Myrtaceae) is an aromatic evergreen shrub or small tree. It is native to the Mediterranean region.

Objectives: This study aimed to assess antifungal activity (in vitro) of the ethanolic extracts of *Myrtus communis* leaves as a growth inhibitor against 24 clinical isolates of *Candida*, including *C. albicans*, *C. glabrata*, and *C. tropicalis* also three species of *Aspergillus*, including *A. niger*, *A. flavus*, and *A. terreus*.

Materials and Methods: The ethanolic extract of myrtle leaves was prepared by maceration method and minimal inhibitory concentration (MIC) of *Myrtus communis* leaves extract was determined by agar-well diffusion technique. Amphotericin B and clotrimazole were used as the positive control in this assay.

Results: The minimal inhibitory concentration (MICs) values of *Myrtus communis* leaves extract ranged 0.625-5.0 µg/µL and 5-40 µg/µL against tested *Candida* spp. and *Aspergillus* spp., respectively.

Conclusions: Results revealed that the ethanolic extract of *Myrtus communis* leaves have antifungal potency against both pathogenic tested fungi, and it can be used as a natural antifungal agent.

Keywords: Myrtus; Aspergillus; Candida; Agar; diffusion

1. Background

According to the World Health Organization (WHO) report, more than 80% of the world's population use traditional medicine for treatment (1). Herbal medicines have been used in Asia for a long time (1). Evaluation of medicinal plants for antimicrobial activity is important for finding new therapeutic compounds. *Myrtus communis* Linn. (Family, Myrtaceae) is an aromatic evergreen shrub or small tree, with 1.8-2.4 m in height. This plant has small foliage and deep fissured bark. It is native to Northern Africa, Southern Europe, and Western Asia and widespread in the Mediterranean region (2). *Myrtus* is a Greek name for myrtle, and *communis* means a plant, which grows in groups (3).

This study was carried out to find a replacement agent for amphotericin B to be used in treatment of mycotic infections. Amphotericin B has been used for the treatment of fungal infections since the 1950s. It has side effects such as hepatotoxicity and nephrotoxicity and often accompanied by azoles (4). *Aspergillus* species are filamentous and commonly isolated from environment. Some *Aspergillus* species such as *A. flavus* cause food contamination (5) and the source of opportunistic infections in human such as aspergillosis (6). Also, *Candida albicans* is responsible for opportunistic infections in oral cavity, digestive tract and vaginal cavity (7). *Candida* species are yeast and the most common cause of fungal opportunistic infections.

Myrtus communis (myrtle) has been recognized in Iran and has several therapeutic properties such as antioxidant (8, 9), antimicrobial (10, 11), anti-hyperglycemic (12), and analgesic effects (13). The antimicrobial activity of myrtle oil was known against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* by Yadegarinia et al. (8). Also, antibacterial property of this plant was reported by Degtyareva et al. (14). Gunduz et al. reported that myrtle oil possess antibacterial action against *Salmonella typhimorium* (15), *Lactobacillus* spp., *Yersinia enterocolitica* (16, 17) and *Helicobacter pylori* (18). Weak antifungal activity of myrtle oil against *Fusarium solani*, *Rhizoctonia solani*, and *Colletotrichum linelemuthianum* was reported (10). Previous studies have revealed the chemical composition of myrtle oil from leaves (8, 19-22).

2. Objectives

The present investigation aimed to assess the antifungal efficacy of the ethanolic extract of *Myrtus communis*.

3. Materials and Methods

3.1. Plant Material

Leaves of *Myrtus communis* L. were obtained from Agri-

cultural and Natural Resources Center, Ahvaz, Iran. They were dried in shade, powdered in miller, and stored in an airtight bottle for future work.

3.2. Plant Extraction

Ten grams of plant powder were soaked in 100 mL of 80% ethanol and kept on a rotary shaker for 3 × 24 hours at room temperature. Then crude ethanol extract was filtered using Whatman filter paper No.1, and the supernatant of extract was evaporated to dryness at room temperature. Dried extract was stored in sterile bottles and kept at -20°C for assay.

3.3. Fungal Isolates and Fungal Suspensions Preparation

Twenty-one *Candida* isolates, including 9 *C. albicans*, 6 *C. glabrata*, and 6 *C. tropicalis* from oral cavity and also one environmental isolate from each of *A. niger*, *A. flavus* and *A. terreus* were tested in this study. The fungi from stock were subcultured on Sabouraud dextrose agar (SDA; Merck, Germany). A loopful of spore from the stock cultures was added to the test tubes of Sabouraud dextrose broth (SDB) and grown overnight on a rotary shaker at room temperature. Then broth cultures were centrifuged at 12000 rpm for 10 minutes, and then the supernatant was discarded. The fungal cells were pelleted by centrifugation washed with phosphate-buffered saline (pH 7.5) three times and fungal suspensions adjusted to 10⁸ colony-forming units (CFU/mL) to obtain the turbidity of 0.5 McFarland by the same buffer (23)

3.4. Positive and Negative Controls

The commercial antifungal drugs such as clotrimazole and amphotericin B (4 µg/µL) were used as positive con-

trol and absolute dimethyl sulfoxide (DMSO) was used as the negative control.

3.5. Determination of minimum inhibitory concentration (MIC)

Aliquots of 1000 mg dried extract were dissolved in 5 mL absolute of DMSO for obtaining concentration of 200 µg/µL as stock solution. Serial dilutions of plant extracts were prepared in a concentration range of 0.156-20 µg/µL according to the previous study (24). The ethanolic extracts of *Myrtus communis* leaves were assayed for antifungal activity using an agar well diffusion method (25). One hundred microliters inoculum (10⁸ CFU/mL) of each tested *Candida* and *Aspergillus* species was uniformly spread by sterile bent glass rod onto SDA medium. The plates were allowed to dry for 5-10 minutes and punched 5 well (7 mm diameter) onto SDA agar medium by using a sterile borer. Subsequently, 100 µL of different concentrations of the ethanolic extracts of *Myrtus communis* leaves were poured into the wells of agar plates. The minimum inhibition concentration (MIC) value was defined as the lowest extract concentration that inhibits the growth at 35°C for 24 hours.

4. Results

Tables 1 and 2 present data for the MIC assay of the tested plant. The strongest activity was shown against *C. glabrata* with the MIC values of 0.625-5.0 µg/µL and the range of 15-30 mm inhibition zones while the highest concentration showed a weak inhibitory effect against *Aspergillus* spp. with the MIC values of 5.0-40 µg/µL. In this research, clotrimazole and amphotericin B were used as positive controls. MIC value of clotrimazole was 0.25 µg/µL and its inhibition zone against *C. albicans* was 18-25 mm. Also, MIC value of amphotericin B was 0.125 µg/µL, and its inhibition zone against *Aspergillus niger* was 17-22 mm as shown in Tables 1 and 2.

Table 1. The MIC values in (µg/µL) of the Ethanolic Extract of *Myrtus Communis* Leaf Against *Candida* spp. and *Aspergillus* spp. Isolates^{a,b,c}

	<i>A. terreus</i> (No.1)	<i>A. flavus</i> (No.1)	<i>A. niger</i> (No.1)	<i>C. tropicalis</i> (No.6)	<i>C. glabrata</i> (No.6)	<i>C. albicans</i> (No.9)
MIC values, µg/µL	20-40	20-40	5.0-40	1.25-5.0	0.625-5.0	2.5-20
Positive controls, µg/µL						
Clotrimazole	-	-	-	-	-	0.25
Amphotericin B	-	-	0.125	-	-	-

^a Abbreviation: MIC, minimal inhibitory concentration.

^b Tests were done in triplicate.

^c Tested concentrations: extracts = 40 µg/µL; Positive Control: Clotrimazole (4 µg/µL); Amphotericin B (4 µg/µL).

Table 2. The inhibition zone (mm) of *Myrtus communis* leaf extract against *Candida* spp. and *Aspergillus* spp. Isolates^a

	<i>A. terreus</i> (No.1)	<i>A. flavus</i> (No.1)	<i>A. niger</i> (No.1)	<i>C. tropicalis</i> (No.6)	<i>C. glabrata</i> (No.6)	<i>C. albicans</i> (No.9)
MIC values, mm	13-21	0-20	0-11	10-25	15-30	7-23
Positive controls, mm						
Clotrimazole	-	-	-	-	-	18-25
Amphotericin B	-	-	17-22	-	-	-

^a Values are the mean of three replicates; Tests were done in triplicate; Tested concentrations: extracts = 40 µg/µL; Positive Control: Clotrimazole (4µg/µL); Amphotericin B (4 µg/µL).

5. Discussion

In the present study, the antifungal efficacy of *Myrtus communis* was assessed against clinical isolates of *Candida* spp. and *Aspergillus* spp. Among the tested fungi, the strongest activity belonged to *C. glabrata* with MIC values of 0.625-5.0 µg/µL. Similar results were reported by other researchers (26). For example, Mahboubi and Ghazian Bidgoli reported that the essential oil of *Myrtus communis* had significant activity against *C. albicans* ($P < 0.01$) (26). In the present study, MIC value of clotrimazole was 0.25 µg/µL for *C. albicans*. Also, the MIC value of amphotericin B was 0.125 µg/µL against *A. niger*. Similar finding reported by Aali et al. that methanolic extract of *Myrtus communis* extract was greater than that of clotrimazole ($P < 0.001$) (27). Martinez et al. reported the leaf extract of *Myrtus communis* was effective on *Fusarium* spp. while, it was ineffective on *Penicillium* (28). While, in another study, Ouelhadj et al. reported the oil of *Myrtus communis* was most active against *Penicillium* spp. and *Aspergillus niger* (59.5 mm and 46 mm), respectively with the lowest MIC value for both pathogens (125 µL/mL) (29).

Also, previous studies revealed that the essential oil of *Myrtus communis* L. was tested in vitro against phytopathogenic fungi such as *Rhizoctonia solani*, *Fusarium solani* and *Colletotrichum lindemuthianum*. The essential oil of *M. communis* inhibited the growth of fungi by 60% in the concentration of 1600 ppm and microscopic observation revealed that the essential oil of *M. communis* caused morphological alterations of hyphae of all fungi (10). Antifungal activity of the methanolic extract of *Myrtus communis* leaves against dematiaceous such as *Alternaria* spp. was reported by Sacchetti et al. (30). Ameziane et al. revealed antifungal activity of the leaf extract of *Myrtus communis* against *Geotrichum candidum* (31). Furthermore, previous studies revealed the essential oil of *Myrtus communis* L. was tested in vitro activity against phytopathogenic fungi such as *Rhizoctonia solani*, *Fusarium solani* and *Colletotrichum lindemuthianum*.

Shahidi Bonjar et al. reported that the essential oil of *Myrtus communis* inhibited the growth of bacteria such as *Staphylococcus aureus*; *Escherichia coli*; *Klebsiella* sp. and fungi such as *Candida albicans* but, was not effective against *Saccharomyces cerevisiae* (32). The anti-*Candida* activity of the leaf extract of *Myrtus communis* was confirmed (33). Miller et al. reported anti-fungal activity of this plant against *Cladosporium* spp. (34). The inhibition efficacy of the leaf extract of *Myrtus communis* in the concentrations of 1%, 10% and 15% against *Salmonella typhimurium*, *Staphylococcus aureus*, *Candida rugosa* and *Aspergillus niger* (35). Other studies showed that this plant has the most efficacy on the mosquito *Culex pipiens* (36) and the plant was effective at pH 6.8 against *Trichomonas vaginalis* but, it was ineffective at pH 6.

In conclusion, the MIC values showed that the tested plant extract besides its antimicrobial effects was quite active against fungi at tested concentrations. Generally,

finding alternative drugs derived from natural agents (herbal sources) is important in the future, because they are safer and cheaper than commercial drugs with respect to their antifungal and antibacterial therapy.

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