Letter to the Editor

Antimycotic activity of *Myrtus communis L.* towards *Candida* spp. from clinical isolates

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Introduction

Myrtle (*Myrtus communis L.*) is an evergreen shrub belonging to the family of Myrtaceae, which grows spontaneously throughout the Mediterranean area. In Italy it grows along the coasts and on the internal hills, and it is abundant especially in the islands, where it is one of the most characteristic species. The essential oil obtained from its leaves and sometimes from its flowers and berries has been used for its tonic and medicinal properties, and it is used in the flavour and fragrance industries. Essential oils are gaining remarkable interest for their potential multi-purpose use as antioxidant, antibacterial and antiseptic agents [1]. Myrtle berries and leaves are mostly employed for the industrial formulation of sweet liquors with digestive properties; however, the essential oil obtained from myrtle leaves has been used in the treatment of lung disorders, and *Myrtus communis* has a history in popular and traditional medicine [2]. *Myrtus communis* has exhibited the biological activities of tannins including anticancer and antioxidant activities [3]. In previous studies, we obtained encouraging results when we evaluated the antimicrobial properties of myrtle essential oil against several clinical strains, in particular against *Helicobacter pylori* and *Mycobacteria* [4,5].

Candidemia is the fourth most common nosocomial bloodstream infection. The incidence of candidemia has risen worldwide over the last two decades in different settings, mostly due to an increase in the use of more aggressive therapy practices, especially intensive chemotherapy for hematological malignancies, transplantation, and intensive care unit (ICU) use. To a lesser extent, the use of immunosuppressive agents for the treatment of autoimmune and other diseases, and even for the prolongation of life, has created a previously non-existent immunocompromised population. *Candida albicans* is the most causative agent involved with fungal infections [6,7], and *Candida* non *albicans* are known to be even more resistant to conventional antifungal therapy. Candidemia is associated with considerable mortality. The management of *Candida* infections faces many problems, such as a limited number of antifungal drugs, toxicity, resistance of *Candida* to commonly used antifungal drugs, relapse of *Candida* infections, and the high cost of antifungal drugs [8,12]. It is therefore essential to discover new antifungal agents to combat strains expressing resistance to the available antifungal drugs. Natural products have been used as therapeutic agents and about half of the drugs that we use today are derived from natural sources [2,13]. Because of the development of microbial resistance, natural products are a valuable source of new antibiotic drugs [2]. Aromatic plants have been used since ancient times for their medicinal properties. Essential oils (EO) are complex mixtures of volatile compounds that result from secondary metabolic pathways of plants [13,14].

The aim of this work was to evaluate the antimycotic activity of myrtle's essential oil toward five species of yeasts isolated from clinical samples (blood cultures): *C. albicans* (10 strains), *C. glabrata* (10 strains), *C. krusei* (10 strains), *C. tropicalis* (10

Table 1. *In vitro* susceptibility of *Candida* spp. isolates to myrtle oil and 6 antifungal drugs

	Myrtle (□l/ml)		Amphotericin B(□g/ml)		Fluconazole (□g/ml)		Voriconazole (□g/ml)		Anidulafungin (□g/ml)		Micafungin (□g/ml)		Caspofungin (□g/ml)	
Organism (n)	Range	MIC_{90}	Range	MIC_{90}	Range	MIC_{90}	Range	MIC_{90}	Range	MIC_{90}	Range	MIC_{90}	Range	MIC_{90}
C. albicans (10)														
24 h	0.5-2	2	0.125-0.5	0.5	0.125-0.5	0.5	0.008-0.03	0.03	0.008-0.03	0.03	0.008-0.03	0.03	0.03-0.125	0.125
48 h	1-2	2	0.25-0.5	0.5	0.25-1	1	0.008-0.03	0.03	0.008-0.03	0.03	0.008-0.03	0.03	0.06-0.125	0.125
C. glabrata (10)														
24 h	0.5-4	4	0.25-1	1	4-128	128	0.06-2	2	0.008-0.03	0.03	0.016-0.06	0.06	0.03-0.125	0.125
48 h	1-4	4	0.5-1	1	8-256	256	0.125-4	4	0.016-0.06	0.06	0.03-0.125	0.125	0.06-0.125	0.125
C. krusei (10)														
24 h	1-4	4	0.125-0.5	0.5	16-64	64	0.03-0.125	0.125	0.016-0.06	0.06	0.03-0.125	0.125	0.06-0.125	0.125
48 h	2-4	4	0.25-1	1	32-128	128	0.06-0.125	0.125	0.03-0.125	0.125	0.06-0.125	0.125	0.125-0.25	0.25
C. tropicalis (10)														
24 h	0.25-1	2	0.125-0.5	0.5	0.5-1	1	0.008-0.03	0.03	0.03-0.06	0.06	0.06-0.125	0.125	0.125-0.25	0.25
48 h	0.5-2	2	0.25-1	1	0.5-2	2	0.016-0.03	0.03	0.03-0.06	0.06	0.06-0.125	0.125	0.125-0.25	0.25
C. parapsilosis (10)	0.5-2	2	0.25-0.5	0.5	0.5-2	2	0.03-0.125	0.125	0.25-1	1	0.25-1	1	0.125-1	1
24 h	10.5-4	4	0.25-1	1	0.5-4	4	0.06-0.25	0.25	0.5-1	1	0.5-2	2	0.25-1	1
48 h														

strains), and *C. parapsilosis* (10 strains). The results obtained at 24 and 48 hours were compared with the MIC using the following six antimycotics: amphotericin B, fluconazole, voriconazole, anidulafungin, micafungin, and caspofungin. The results obtained showed good antimycotic activity of myrtle's essential oil toward the five yeasts at the 24- and the 48-hour time points.

The study

In this study the isolation of essential oils from *Myrtus communis* leaves was obtained by a hydrodistillation method using a Clevenger-type apparatus, according to the Italian Official Pharmacopoeia. The composition of the essential oils was analysed by GC/MS. A collection of 50 isolates belonging to five different species of Candida spp. was selected for this study: *C. albicans* (10), *C. glabrata* (10), *C. krusei* (10), *C. tropicalis* (10), and *C. parapsilosis* (10). The isolates were cultured from blood samples of hospitalized patients at the Institute of Microbiology Policlinico A. Gemelli in Rome. All microorganisms were identified by standard methods (germe tube test) and stored on Sabouraud dextrose agar plates until the study was performed [15]. The minimum inhibitory concentration (MIC) of myrtle oil was determined using the M27-A3 method [16], appropriately modified. Yeasts were cultivated at 37°C on Sabouraud agar plates (Kima, Padova, Italy) for 24 hours. The inoculum was prepared by a dilution of the colonies in salt solution, at a 0.5 McFarland concentration, and the concentration was confirmed by a spectrophotometric reading at a wavelength of 530 nm. The sensitivity test was performed in RPMI 1640, using 96-well plates. Myrtle oil concentrations were prepared by serial one to two dilutions from 32 µg/ml to 0.06 µg/ml. After shaking, 100 µl of each oil dilution and 100 µl of yeast suspension were added to each well and then incubated at 35°C for 48 hours. MIC was defined as the lowest concentration able to inhibit the fungal growth of 50% compared to the growth control and MIC values registered at 24 and 48 hours. Each isolate was tested in duplicate. Furthermore, each yeast strain included in the study was tested for its sensitivity to amphotericin B, fluconazole, voriconazole, caspofungin, anidulafungin, and micafungin following the M27-A3 protocol and using the CLSI breakpoints to establish susceptibility/resistance of the studied isolates [16]. Quality control strains (*Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258) were incorporated in each set of experiments.

Results

The MIC data for the *Candida* spp. are summarized in the Table. After 24 hours the ranges of MIC obtained with myrtle essential oil were 0.5-2 µg/ml, 0.5-4 µg/ml, 1-4 µg/ml, 0.25-1 µg/ml, and 0.5-2 µg/ml for *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. parapsilosis* respectively; MIC90 were 2 µg/ml for *C. albicans*, *C. tropicalis* and *C. parapsilosis*, while MIC90 for *C. glabrata* and *C. krusei* were 4 µg/ml. After 48 hours the ranges of MIC were 1-2 µg/ml for *C. albicans*, 1-4 for *C. glabrata*, 2-4 for *C. krusei*, 0.5-2 µg/ml for *C. tropicalis* and 0.5-4 µg/ml for *C. parapsilosis*. MIC90 were 2 µg/ml for *C. albicans*, *C. tropicalis*, and 4 µg/ml for *C. glabrata*, *C. krusei* and *C. parapsilosis*. Furthermore, *C. glabrata* and *C. krusei* were resistant to fluconazole (MIC90: 128 and 64 µg/ml respectively after 24 hours and 256 and 128 µg/ml after 48 hours), and *C. glabrata* was resistant to voriconazole (MIC90: 2 µg/ml after 24 hours and 4 µg/ml after 48 hours). All the yeasts tested were susceptible to amphotericin B, anidulafungin, micafungin and caspofungin.

Discussion

The aim of this study was to find an alternative for antifugal drugs currently used in the treatment of fungal infections. Since it has been demonstrated that myrtle essential oil has an antibacterial activity towards *Escherichia coli*, *Staphylococcus aureus*, *Helicobacter pylori* and other Gram-negative bacteria, we decided to test its antifungal activity against different species of *Candida*. The results obtained show good activity of the extract against *C. albicans* and *C. tropicalis* after 24 to 48 hours, and against *C. parapsilosis* after 24 hours. Good antifungal activity was also seen after 48 hours against *C. glabrata*, *C. krusei* and *C. parapsilosis*, but with higher MIC. At first sight, the results obtained seem higher than those obtained with the antimycotic tested, but lower than those reported in literature [17]. In particular, Mahboubi and colleagues obtained MIC values regarding *C. albicans* three times higher than those we registered [17]; however, Mahboubi's group studied the essential oil of Turkish myrtle, which differs from Italian myrtle essential oil. The essential oil of Turkish myrtle oil presents a higher percentage of linalool and linalyl acetate compared with Italian myrtle oil, the main components of which are α pinene and 1.8 cineole. Therefore, it is possible to conjecture that the different activity of myrtle essential oil is due to their different percentage compositions. Our work included not only *C. albicans*, but also *C.* non *albicans* strains,

with promising results. These data are very important considering that some yeasts, such as *C. glabrata* and *C. krusei*, are resistant to conventional antifungal drugs, making them difficult to treat. Further studies are necessary to evaluate myrtle essential oil's exact chemical composition and toxicity.

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