

Antimicrobial Activities of Essential Oils against Common Hospital Fungi Species

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Abstract

Introduction: In hospitals and other healthcare settings the presence of airborne and sedimented fungi is an extrinsic risk factor for opportunistic infections involving both immunocompromised and non-immunocompromised persons. In hospitalized patients, it is estimated that 9% of hospital-acquired infections are caused by fungi. Lethality rate varies from 40% to 100% depending on the immunosuppression degree of stakeholders. To prevent healthcare-associated infections, the control of environmental fungal contamination through use of sanitizing/disinfecting practices is basic. However, the widespread use of common disinfectants could promote the growth of antibiotic-resistant superbugs and cause environmental harm. These aspects stimulated the search of new antimicrobial agents. The aim of this study was to evaluate the antimicrobial activity of essential oils of *Mentha insularis* Req., *Mentha pulegium* L., *Mentha requienii* Benth, *Artemisia caerulea* L. ssp. *densiflora* (Viv), *Rosmarinus officinalis* L. var. *albiflorus*, *Rosmarinus officinalis* L. var. *lavandulifolius*, and *Ocotea puchury major* Mart. against fungi species frequently found in hospitals and potentially responsible for opportunistic mycoses. **Methods:** The essential oils' antifungal activity was carried out by agar disc diffusion technique. **Results:** All tested essential oils are effective, though to a different degree, against both molds that yeasts assessed. The major antifungal activity was showed by *Mentha* oils. Particularly, *Mentha requienii* and *Mentha insularis* oils were active until 1:8 dilution against *Rhodotorula* spp. and 1:16 dilution against mixed molds, while *M. pulegium* was strongly active until 1:2 against both fungi. **Conclusions:** To the best of our knowledge, few or no data are available in literature on the activity of essential oils against hospital environmental isolates of fungi. Results suggest their potential application in sanitation procedures of the hospital, and in general, of the "care settings".

Keywords

Hospital Environment, Opportunistic Mycoses, Essential Oils, Antifungal Activity

1. Introduction

Fungi, previously considered to be of low virulence or non-pathogenic, can cause serious health problems in susceptible people [1]-[4]. The environment has been suggested as playing a crucial role in the source of these problems [5] [6]. In particular, in hospitals and other healthcare settings the presence of airborne and/or sedimented fungi is an extrinsic risk factor for opportunistic infections, as demonstrated, in several studies, by the correspondence between the genotypic fungal species found in air and surfaces and the one responsible for hospital infection [5] [7]-[9]. However, nowadays the possibility of fungal pollution in hospital is a particularly serious problem in relation to the building works in and around hospitals that, for adaptation of health facilities with the most modern care needs, is affecting different health realities. Site renovation and construction can disturb fungi-contaminated dust and promote spread of fungal spores in the hospital environment. These can contaminate indoor air and surfaces, thus implementing the risk of invasive fungal infections as well as allergic reactions in fungal-sensitive individuals [8] [10] [11]. Moreover, aging population and survival of patients with serious diseases, facilitated by advances in diagnosis and treatment, determine the increase of susceptible subjects and, consequently, the likelihood of infection and the severity of clinical pictures. In severely immunocompromised individuals (e.g. patients with hematological malignancies, solid organ and bone marrow transplant recipients, cancer patients undergoing radiotherapy or chemotherapy, patients with full-blown AIDS), fungal infections are systemic, rapidly progressive, and difficult to diagnose or treat [2] [12]. Furthermore, the risk of fungal infection involves also non-immunocompromised persons, particularly those who have just undergone surgery with prolonged exposure of deep tissues or those with burns [1] [13].

The incidence of fungal infections is increasing over the years [4] [12]. Epidemiological data show an incidence of fungal infections more than 1% in institutionalized subjects [14]; considering only hospitalized patients, it is estimated that 9% of hospital-acquired infections are caused by fungi [3]. Lethality rate varies from 40% to 100% depending on the immunosuppression degree of stakeholders [8] [14] [15].

To prevent healthcare-associated infections, the control of environmental fungal contamination through use of sanitizing/disinfecting practices, able to reduce amount and variety of fungi which may come into contact with patients, is basic. Extensive use of chemical disinfectant agents is essential in healthcare; however, it could cause micro-organisms to become resistant to disinfectants as well as to antibiotics as also highlighted by a recent study which has provided more evidence that using common disinfectants could promote the growth of antibiotic-resistant superbugs [16]-[19]. The widespread use of disinfectant also could cause environmental harm. These aspects stimulate the search to discover new environmentally friendly antimicrobials to contribute to the control of microbial resistance.

In this regard, natural products, either pure compounds or standardized plant extracts, provide many opportunities. The antimicrobial properties of aromatic and medicinal plants and their extracts have been recognized since antiquity. In particular, essential oils (EOs), formed by aromatic plants as secondary metabolites, are known, since ancient times, for their antiseptic and medicinal properties, and their fragrance. They can be extracted from plant material (*i.e.* flowers, leaves, buds, seeds, twigs, stems, fruits, bark, herbs, wood or roots) by expression, fermentation, enfleurage, extraction or steam distillation. In nature, EOs play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and also against herbivores by reducing their appetite for such plants. For their properties observed in nature, since the middle ages, EOs have been largely employed and nowadays some of them are used in pharmaceutical, sanitary, agronomic, food, cosmetic and perfume industries as alternatives to synthetic chemical products to protect the ecological equilibrium, and as natural remedies [20] [21].

Since a few years, our research group has undertaken studies on the antibacterial and antifungal properties of essential oils in order to find their possible new applications.

The aim of this study was to evaluate the antimicrobial activities of seven essential oils against fungi species frequently found in hospitals and potentially responsible for opportunistic mycoses, in order to check the possi-

bility of using them in the practices of hospital sanitation as alternative or adjuvants to the use of synthetic chemicals.

2. Methods

For antimicrobial activity, were screened essential oils of *Mentha insularis* Req., *Mentha pulegium* L., *Mentha requienii* Bentham, *Artemisia caerulescens* L. ssp. *densiflora* (Viv), *Rosmarinus officinalis* L. var. *albiflorus*, *Rosmarinus officinalis* L. var. *lavandulescens*, and *Ocotea puchury mayor* Mart.

In almost all cases, essential oils was obtained from fresh material collected in different areas of Sardinia; for *Ocotea puchury mayor* Mart. we started from dry leaves harvested in the Amazonian rain forest of Borba District (Brazil) along the edge of Madeira River.

The plants from Sardinia were identified following the keys reported in Flora d'Italia [22] by Mario Chessa from University of Sassari and a representative specimen of the species was deposited in the Herbarium "SASSA" in the Department of Chemistry and Pharmacy of University of Sassari at the numbers 1073, 1072, 1074, 736, 1091 and 514 for *Mentha insularis* Req., *Mentha pulegium* L., *Mentha requienii* Bentham, *Artemisia caerulescens* L. ssp. *densiflora* (Viv), *Rosmarinus officinalis* L. var. *albiflorus*, *Rosmarinus officinalis* L. var. *lavandulescens*, respectively. Botanical characterization of *Artemisia caerulescens* L. ssp. *densiflora* and the identification of collection sites was also supported by the work of B. Corrias [23].

For *Ocotea puchury mayor* Mart., the identification was based on works by Rohwer [24] and Van der Verff [25]. Voucher samples of the studied parts of plant material are deposited at the Pharmaceutical Botany Laboratory in the Department of Environmental Biology, Museo Erbario, University Sapienza of Rome.

In Department of Chemistry and Pharmacy, University of Sassari, Italy, aerial parts plants were steam distilled for three hours using a Clevenger-type system (European Directorate for the Quality of Medicines, 1975). Essential oils were dried with anhydrous sodium sulfate and kept in amber vials at 4°C until chemical characterization and antimicrobial assay.

The GC analyses were carried out using a Varian 3300 instrument equipped with a FID and a HP-5 capillary column (30 m × 0.25 mm, 0.25 µm film thickness) or HP InnoWax capillary column (30 m × 0.25 mm, film thickness 0.17 µm).

GC/MS analyses were carried out using a Hewlett Packard 5890 GC/MS system operating in the EI mode at 70 eV, using the above mentioned capillary column.

The identification of the components was made for both the columns, by comparison of their retention indices and mass spectra with those of commercial (NIST 98 and WILEY) and home-made library mass spectra built up from pure compounds and MS literature data. Area percentages were obtained electronically from the GC-FID response without the use of an internal standard or correction factors.

In Department of Biomedical Sciences, Hygiene and Preventive Medicine, University of Sassari, the essential oils antifungal activity was carried out by agar disc diffusion technique (aromatogram) against yeasts and moulds: *Rhodotorula* spp., *Candida* spp., *Aspergillus fumigatus*, *Fusarium* spp., *Alternaria* spp. and a mixture of moulds (mixed moulds). All test organisms were obtained from air and surfaces of healthcare facilities. The assay against mixed moulds and *Rhodotorula*, which are the environmental isolates most frequently found, was also performed on the oils scalar dilutions in *n*-hexane ranging from 1:1 to 1:64. Preliminarily, the antifungal activity of the solvent was assessed against the same test organisms; no activity was showed.

Fresh fungal cultures were suspended in sterile saline solution to obtain a final concentration standardized with 0.5 MacFarland scale. 0.1 ml of fungal suspensions was seeded by spreading in Sabouraud Dextrose Agar and filter sterile 6-mm paper disks containing 15 µl of each essential oil was applied to the surface of Sabouraud Dextrose Agar plates to be tested. A "clean" paper disk (no oil) was used as a positive control for growth of fungi. Afterward, the plates were incubated for 24 - 48 h at 30°C [26]-[29].

Essential oils activity was evaluated by measuring the area around the disk where growth of the microorganism is prevented (inhibition zone). The scale of measurement was the following (disk diameter included): ≥20 mm zone of inhibition is strongly inhibitory; <20 - 12 mm zone of inhibition is moderately/mildly inhibitory; and <12 mm is noninhibitory [26]. Tests were carried out in triplicates; mean values and standard deviations were calculated.

3. Results

As can be seen from **Table 1**, prediluted oils from the three *Mentha* species, *Artemisia caerulescens* L. ssp. *den-*

Table 1. Zones of growth inhibition in mm (average \pm standard deviation) showing antifungal activity for pre-diluted essential oils.

| | <i>Aspergillus fumigatus</i> | <i>Rhodotorula</i> spp. | <i>Candida albicans</i> | Mixed moulds | <i>Fusarium</i> spp. | <i>Alternaria</i> spp. |
|---|------------------------------|-------------------------|-------------------------|-----------------|----------------------|------------------------|
| <i>Mentha insularis</i> Req. | 59.7 \pm 2.52 | 55.0 \pm 3.00 | 50.0 \pm 10.00 | 69.3 \pm 4.04 | 42.7 \pm 1.53 | 31 \pm 1.00 |
| <i>Mentha pulegium</i> L. | 55 \pm 2.00 | 50.7 \pm 3.06 | 50 \pm 10.00 | 70 \pm 0.58 | 41.3 \pm 1.53 | 31 \pm 1.00 |
| <i>Mentha requienii</i> Bentham | 60.7 \pm 2.31 | 45.0 \pm 4.00 | 40.3 \pm 3.51 | 71.3 \pm 2.31 | 41.3 \pm 0.58 | 41.7 \pm 1.53 |
| <i>Artemisia caerulescens</i> L. ssp. <i>Densiflora</i> (Viv) | 32 \pm 4.00 | 34.7 \pm 0.58 | 23 \pm 2.00 | 32 \pm 2.52 | 30 \pm 1.53 | 33.3 \pm 0.58 |
| <i>Ocotea puchury mayor</i> Mart. | 64.3 \pm 5.13 | 37.3 \pm 1.15 | 33.3 \pm 9.61 | 39.3 \pm 1.15 | 31 \pm 2.65 | 31.7 \pm 2.08 |
| <i>Rosmarinus officinalis</i> L. var. <i>albiflorus</i> | 35.3 \pm 4.51 | 20.3 \pm 0.58 | 24 \pm 8.72 | 15.7 \pm 1.15 | 24 \pm 0.58 | 31.7 \pm 1.15 |
| <i>Rosmarinus officinalis</i> L. var. <i>lavandulescens</i> | 56.3 \pm 5.13 | 30.0 \pm 3.00 | 34 \pm 8.54 | 31 \pm 1.00 | 31.7 \pm 1.53 | 32 \pm 2.65 |

siflora (Viv), *Ocotea puchury mayor* and *Rosmarinus officinalis* L. var. *lavandulescens* showed the major effectiveness, evidenced by zones of growth inhibition by far >20 mm against all tested fungi. Particularly, the antifungal activity of *Mentha* spp. oils always determined the presence of inhibition zone diameter at least twice that required for strongly inhibitory activity (inhibition zone diameter ranged from >40 to >70 mm). *Rosmarinus officinalis* L. var. *albiflorus* oil was strongly inhibitory against yeasts and isolated moulds, but mildly inhibitory against mixed moulds (15 mm inhibition zone).

As regards diluted oils, whose action has been evaluated only in relation to mixed molds and *Rhodotorula* spp. considered to be the most common environmental fungal isolates, *Mentha* oils showed the major antifungal activity. Particularly, *Mentha requienii* and *Mentha insularis* oils were active (from strongly to mildly with increasing dilution) until 1:8 dilution against *Rhodotorula* spp. and 1:16 dilution against mixed molds, while *M. pulegium* was strongly active until 1:2 against both fungi. *Ocotea puchury mayor* and *Artemisia caerulescens* L. diluted oils had inhibitory activity even at a dilution of 1:2 against mixed molds and of 1:2 and 1:1 against *Rhodotorula* spp., respectively. *Rosmarinus officinalis* L. var. *lavandulescens* diluted oil showed mildly activity against mixed molds at the dilution of 1:1, while *Rosmarinus officinalis* L. var. *albiflorus* was no active.

4. Discussion

Currently, hospitals are facing with an increasing risk of opportunistic mycoses, as a result often ominous, conditioned by particular and critical features, previously highlighted, of guest/environment interaction. Therefore, for prevention, it is basic that contamination of the environment is minimized through the containment of the concentration of fungi within values capable of ensuring a relative safety against the risk of onset of disease.

For this purpose, between the measures of proven effectiveness is the correct and continuous environmental sanitation/disinfection. Since the widespread use of chemical disinfectants could cause the emergence of disinfectants as well as antibiotics resistance, there is a need to research new antimicrobials agents. In this context, the possibility of using in this practice essential oils as an alternative to traditional disinfectants is very interesting.

It is known that many essential oils have antibacterial and antifungal properties [20] [21], however, to the best of our knowledge, few or no data are available in literature on the activity of essential oils against hospital environmental isolates of fungi. Particularly, the antifungal activity of seven essential oils (*M. insularis* Req., *M. pulegium* L., *M. requienii* Bentham, *A. caerulescens* L. ssp. *densiflora* (Viv), *R. officinalis* L. var. *albiflorus*, *R. officinalis* L. var. *lavandulescens*, and *O. puchury mayor* Mart.) was evaluated *in vitro* against fungi isolated from air and surfaces of healthcare facilities (Table 2).

The results showed that the tested plant essential oils have a different degree of antimicrobial activity against mould and yeast considered. *In vitro* antifungal activity of the pre-diluted essential oils ranged from strongly to moderate inhibitory activity; however, oils extracted from plants belonging to the same genus showed an almost uniform behavior against all tested fungi. Moreover, because aspergilloses are the most common form of opportunistic filamentous mycoses in immunocompromised persons, particularly interesting is the finding that these

Table 2. Zones of growth inhibition in mm (average \pm standard deviation) showing antibacterial activity for diluted essential oils.

| Oils | <i>Rhodotorula</i> spp. | | | | | | |
|---|-------------------------|-----------------|-----------------|-----------------|-----------------|------|------|
| | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 |
| <i>Mentha insularis</i> Req. | 41.3 \pm 7.51 | 33.7 \pm 0.58 | 29 \pm | 12 \pm | n.a. | | |
| <i>Mentha pulegium</i> L. | 51.0 \pm 11.00 | 45.7 \pm 6.03 | n.a. | | | | |
| <i>Mentha requienii</i> Bentham | 40.0 \pm 1.00 | 33.7 \pm 7.51 | 26.7 \pm 2.89 | 16.0 \pm 5.20 | n.a. | | |
| <i>Artemisia caerulescens</i> L. ssp. <i>densiflora</i> (Viv) | 12.0 \pm 1.00 | n.a. | | | | | |
| <i>Ocotea puchury mayor</i> Mart. | 31.3 \pm 9.71 | 14.7 \pm 6.43 | n.a. | | | | |
| <i>Rosmarinus officinalis</i> L. var. <i>albiflorus</i> | n.a. | | | | | | |
| <i>Rosmarinus officinalis</i> L. var. <i>lavandulescens</i> | n.a. | | | | | | |
| Oils | Mixed moulds | | | | | | |
| | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 |
| <i>Mentha insularis</i> Req. | 68.3 \pm 5.69 | 50.3 \pm 1.53 | 28.7 \pm 1.15 | 23.7 \pm 1.15 | 15.3 \pm 3.06 | n.a. | |
| <i>Mentha pulegium</i> L. | 54.7 \pm 8.08 | 39.7 \pm 9.29 | n.a. | | | | |
| <i>Mentha requienii</i> Bentham | 68.0 \pm 6.00 | 50.0 \pm 5.00 | 29.7 \pm 2.08 | 23.0 \pm 3.00 | 16.3 \pm 1.53 | n.a. | |
| <i>Artemisia caerulescens</i> L. ssp. <i>Densiflora</i> (Viv) | 31.7 \pm 2.52 | 27.7 \pm 1.53 | n.a. | | | | |
| <i>Ocotea puchury mayor</i> Mart. | 15.3 \pm 2.52 | 12.7 \pm 3.06 | n.a. | | | | |
| <i>Rosmarinus officinalis</i> L. var. <i>albiflorus</i> | n.a. | n.a. | | | | | |
| <i>Rosmarinus officinalis</i> L. var. <i>lavandulescens</i> | 12.0 \pm 2.65 | n.a. | | | | | |

n.a.: no activity.

essential oils inhibit the growth of *Aspergillus fumigatus* the most common cause of human aspergilloses, being responsible for about 90% of cases [15].

The antifungal activity highlighted in the tested essential oils suggests their potential use in the formulation of products designed for sanitation/disinfection procedures of the hospital and, in general, of the “care settings”, resulting in less use of chemical disinfectants. In this way, the essential oils can be also a tool to reduce the development and spread of microbial resistance.

Furthermore, in order to optimize the health and environmental comfort, is not to overlook the fact that the oils usually have a pleasant aroma. Therefore, using oils as an alternative to common disinfectants, which often have unpleasant odors and aggressive that contribute in an important way to determine the classic “hospital smell”, it could foster a perception of more peaceful place for patients and their families and create a more pleasant working environment for health professionals.

5. Conclusions

The aim of this study was to evaluate the antimicrobial activity of seven plant essential oils against fungi species frequently isolated in hospital environment, in order to find environmentally friendly antimicrobials for use in the practices of sanitation.

All tested essential oils were effective, though to a different degree, against both molds that yeasts assessed.

In our opinion, the results suggest their possible use as an alternative or complements to synthetic compounds, without showing the same secondary effects. Further studies are necessary to explore the efficacy of suitable concentrations of these essential oils also against other microorganisms frequently found in hospitals and to establish the technical feasibility of their use as natural sanitizing agents.

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Authors' Contributions

All of the authors participated in planning and design of the study, and all read and approved the manuscript. AP, ABM and MDM participated to define protocols for the essential oils antifungal activity valuation. MC, GP, MF and GP selected the plants and carried out the chemical characterization. SD and MGD performed the antifungal activity tests. AP, GM, GP and MDM conceived the study, participated in its design and wrote the manuscript.

Competing Interests

The authors declare that they have no competing interests.

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