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Short Communication

Effect of Plant Oils on *Candida albicans*

Vishnu Agarwal*, Priyanka Lal, Vikas Pruthi

Department of Biotechnology, Indian Institute of Technology, Roorkee, India.

BACKGROUND/PURPOSE: *Candida* species, notably *Candida albicans*, is the major fungal pathogen in humans. It is a dimorphic fungus capable of causing superficial mucosal infections, as well as systemic infections, in immunocompromised individuals. The factors responsible for its pathogenesis are still not fully understood and increasing resistance to commonly used antifungal agents necessitates the search for new formulations.

METHODS: The inhibitory effect of 30 different plant oils on *Candida albicans* isolated from clinical samples was evaluated. The antifungal agent fluconazole was used as a positive control. Plant oils were tested at concentrations from 0.03% to 3% (v/v) to determine the minimum inhibitory concentration and minimum fungicidal concentration (MFC) using agar dilution and macro broth dilution assays.

RESULTS: Of the 30 plant oils tested, 18 were found to be effective and 12 were ineffective. Based on their MFCs, effective oils were placed into three categories: most effective, moderately effective and least effective. Eucalyptus and peppermint oils were most effective, with MFC values of 0.12% and 0.15% (v/v), respectively.

CONCLUSION: The significant antifungal activity of these oils suggests that they could serve as a source of compounds with therapeutic potential against *Candida*-related infections.

KEYWORDS: *Candida albicans*, fluconazole, infections, plant oils

Introduction

Azole drugs and their derivatives continue to dominate as the antifungal agents of choice against *Candida*-related infections, as either topical applications or oral drugs. Even though they are widely acclaimed for their efficacy, these drugs are known to have side effects.[1,2] Fluconazole, commonly used to treat various *Candida albicans* infections, is fungistatic in nature and there are reports of emerging resistance among clinical isolates of *C. albicans*.[3] Therefore, there is a need to isolate new antifungal agents, mainly from plant extracts, with the goal of discovering new chemical structures without the above disadvantages.[4] Many plant extracts and essential oils have biological activity both *in vitro* and *in vivo*, which has justified research on traditional medicine focused on the characterization of their antimicrobial activity.[5] The antimicrobial activity shown by plant oils is mainly due to a number of phenolic and terpenoid compounds, which have antibacterial or antifungal activity.[6] In addition, it is expected that plant compounds with target sites other than those currently

*Corresponding author. Department of Biotechnology, Motilal Nehru National Institute of Technology, Allahabad, India.

E-mail: vishnu_agarwal02@rediffmail.com or vishnua@mnnit.ac.in

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used by antimicrobials will be active against drug-resistant microbial pathogens. Yet, the information available regarding plants (particularly medicinal plants) that are active against this microorganism has, until recently, not resulted in effective formulations for human use. For this reason, the present study assessed 30 plant oils for their effect against *C. albicans* by standard disc diffusion assay followed by the determination of their minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values by agar dilution and macro broth dilution assays. The plant oils tested were: clove, tulsi, ginger grass, tea tree, ocimum tenuiflorum, castor, juniper, malkangani, coconut, peppermint, babchi, mahua, ginger, mustard, rose oil, jasmine, eucalyptus, lavender, linseed, neem, chamomile, sesame, jyotishmati, jojoba, walnut, almond, khus, wheatgerm, chaulmoogra and cade oil.

Methods

Microorganism and culture conditions

C. albicans previously isolated from clinical samples was used in this study.[7] The fungus was cultured in yeast peptone dextrose broth (YPD, Himedia, India) containing 20 g/L peptone, 10 g/L yeast extract and 20 g/L dextrose and incubated for 24 hours at 35°C with agitation (120 rpm). Following incubation, cells were sedimented by centrifugation (5000 ×*g* for 15 min at 4°C), washed twice with sterile phosphate buffered saline (PBS, pH 7.2) and finally resuspended in PBS (pH 7.2). The culture was appropriately diluted to obtain an inoculation level of 5×10^6 cfu/mL.

Chemicals

Castor, eucalyptus and peppermint oils were purchased from Himedia chemicals (Himedia, India), while the other 27 plant oils—almond, alsi, babchi, babuna, cade, chaulmoogra, clove, coconut, ginger grass, ginger, jasmine, jojoba, juniper, jyotishmati, khus, lavender, mahua, malkangani, mustard, neem, ocimum, rose, tea tree, til, tulsi, walnut and wheatgerm—were obtained by steam distillation.[8]

Screening of plant oils for anti-Candida activity

The anti-*Candida* activity of the 31 plant oils was tested using a standard disc diffusion assay.[9] Yeast extract peptone agar plates were prepared and aseptically spread with 50 µL of 5×10^6 cfu/mL *C. albicans* culture. Plant oils (5 µL) were spotted onto 5-mm discs, transferred to the seeded plates and incubated at 35°C. The diameter of the zone was measured after 48 hours.

Determination of the MIC of plant oils

The MIC of the plant oils was determined using an agar dilution assay.[9] Agar plates were prepared in triplicate by adding yeast extract peptone agar containing different concentrations of plant oils (0.03–3% v/v). Tween-20 (0.5% v/v) was added to enhance the oil solubility. The plates were inoculated with 10^4 cfu using an inoculum of *C. albicans* prepared as described above and incubated for 48 hours at 35°C. Plates with Tween-20 but without any plant oil were used as controls. The number of colonies was counted after 48 hours. The lowest concentration of plant oil preventing visible growth of *C. albicans* was designated as the MIC.

Determination of the MFC of plant oils

The MFC of the plant oils was determined using a macro broth dilution assay.[10] A range of concentrations (0.03–3% v/v) were prepared in YPD broth medium in flasks. Tween-20 was included at a final concentration of 0.5% (v/v) to enhance oil solubility. Each flask was inoculated with 2.5×10^3 cfu/mL *C. albicans*. Flasks containing Tween-20 but lacking any plant oil were used as controls. The flasks were incubated at 35°C, in an orbital shaking incubator (120 rpm) for 48 ± 2 hours. From each flask 50 µL of culture was inoculated onto YPD plates and incubated at 35°C for 48 ± 2 hours. The plates were observed and the MFC was determined as the lowest concentration of plant oil completely inhibiting the growth of *C. albicans*.

Results

Screening of plant oils for anti-Candida activity

Biofilm-forming clinical isolates of *C. albicans* showed different sensitivity to the tested oils. The majority of the oils were effective and showed anti-*Candida* activity even at very low concentrations. Eighteen of the 30 oils tested produced a 1–30-mm zone of inhibition (ZOI). Eucalyptus oil and peppermint oil resulted in 26.7- and 22.2-mm ZOI, four oils showed 10–20-mm ZOI, 12 oils showed 1–10-mm ZOI and 12 oils were not effective, showing no ZOI (Table).

Determination of MIC of plant oils against C. albicans

The MICs of the 18 effective oils and fluconazole against biofilm-forming *C. albicans* were determined. The oils exhibited concentration-dependent inhibition of growth. Fluconazole (4 μg/mL) completely inhibited the growth of *C. albicans*. Eucalyptus oil was the most effective, with complete inhibition occurring at 0.05%. Five oils completely inhibited the growth of *C. albicans* at concentrations ranging from 0.08% to 1.0%. Nine oils were inhibitory at concentrations ranging from 1% to 3%, while rose, jasmine and lavender oils were effective at concentrations > 3.0% (Table).

Determination of MFC of plant oils against C. albicans

The MFC is defined as the lowest concentration of oil resulting in the death of 99.9% of the inoculum. In general, MFC values were greater than their respective MIC values. Based on the MFC values, effective oils could be placed into three classes: oils having an MFC of 0.05–0.15% were considered to be the most effective; oils with MFC values

Table. Classification of plant oils on the basis of zone of inhibition, minimum inhibitory concentration and minimum fungicidal concentration against *C. albicans*

Group	Botanical name	Plant oils	ZOI (mm)	MIC (%)	MFC (%)
Most effective (0.05–0.15%)	*Eucalyptus globulus*	Eucalyptus	26.7	0.05	0.12
	Mentha piperita	Peppermint	22.2	0.08	0.15
Moderately effective (0.15–1.00%)	*Cympopogan martini*	Ginger grass	16.0	0.08	0.29
	Eugenia caryophyllus	Clove	13.8	0.33	0.48
	Ocimum sanctum	Tulsi	11.3	0.48	0.65
	Melaleuca alternifolia	Tea tree	11.0	0.73	0.86
Less effective (> 1.00%)	*Ocimum basiliscum*	Ocimum	9.8	1.50	2.50
	Ricinus Communis	Castor	7.8	2.00	3.00
	Juniperus Chinensis	Juniper	5.6	2.00	2.50
	C. anthelminticum	Malkangni	5.3	1.00	2.00
	Cocos nucifera	Coconut	4.0	1.50	3.00
	Psoralea corylifolia	Babchi	3.4	2.50	2.50
	Madhuca indica	Mahua	3.2	2.50	3.00
	Z. officinalis	Ginger	2.6	3.00	> 3.00
	Brassica juncea	Mustard	2.3	3.00	> 3.00
	R. officinalis	Rose	2.1	> 3.00	> 3.00
	Jasminum nudiflorum	Jasmine	1.4	> 3.00	> 3.00
	Lavandula angustifolia	Lavender	1.2	> 3.00	> 3.00
Non-effective	*Linum usitatissimum*	Alsi	0	–	–
	Azadirachta indica	Neem	0	–	–
	Matricaria chamomilla	Babuna	0	–	–
	Sesamum indicum	Til	0	–	–
	Celastrus paniculata	Jyotishmati	0	–	–
	Simmondsia chinensis	Jojoba	0	–	–
	Juglans regia	Walnut	0	–	–
	Prunus glandulosa	Almond	0	–	–
	Triticum vulgare	Wheatgerm	0	–	–
	Vetiveria zizanoides	Khus	0	–	–
	Juniperus oxycedrus	Cade	0	–	–
	Taraktogenos kurzli	Chaulmoogra	0	–	–
	Fluconazole*			4.00	5.40

*Values in μg/mL. ZOI = zone of inhibition; MIC = minimum inhibitory concentration; MFC = minimum fungicidal concentration.

ranging from 0.15% to 1.00% were moderately effective; and oils with an MFC > 1.00% were less effective. Fluconazole was used as the standard and its MFC was 4.5 µg/mL. Eucalyptus and peppermint oils were most effective with fungicidal values of 0.12% and 0.15%, respectively. Four oils (ginger grass, clove, tulsi and tea tree) were moderately effective, with MFC values ranging from 0.15% to 1.00% (v/v). Twelve oils were less effective, with concentrations > 1.00% being required to elicit a fungicidal effect. MFC values for these oils ranged from 1.00% to 3.00%, except for rose, jasmine and lavender oils, which were not effective, even at concentrations up to 3.0 % (v/v) (Table).

Discussion

Plant oils used as cooking and flavoring agents are increasingly claimed to have broad spectrum antimicrobial activity. Selected oils have been suggested to have potent antimicrobial activity against skin infections, insect bites, chicken pox, colds, flu, measles, sinus congestion, asthma, bronchitis, pneumonia, tuberculosis and cholera, probably due to their phenolic, alcoholic and terpenoid constituents.[1,2] However, azole antifungal agents and their derivatives continue to dominate as the drugs of choice for treating *Candida* infections as either topical applications or oral drugs.[11,12] The present study was undertaken with the prime objective of assessing the antifungal properties of selected plant oils against *C. albicans*. Fluconazole, commonly used against *Candida* infections, was chosen as the control in the study and showed MIC and MFC values of 4.0 µg/mL and 4.5 µg/mL, respectively.

We studied the efficacy of 30 plant oils for their *in vitro* activity against *C. albicans* isolated from clinical samples (Table).[7] These oils were classified into three categories according to their MFCs: most effective, moderately effective and least effective (Table). Eighteen of the selected plant oils were found to be effective. Our results clearly demonstrate that peppermint, eucalyptus, ginger grass and clove oils not only act as potent antifungal agents against *C. albicans*, but also perform better than fluconazole.

The disc diffusion assay is a standard method widely used for the rapid screening of natural products for antifungal activity.[9] Plant oils were screened using this very convenient assay method. The results indicate that caution is needed, since different oils may have different diffusion rates on agar plates, and this may contribute to variations in the size of the inhibitory zones, leading to erroneous conclusions regarding their antifungal activity. For example, some oils that exhibited smaller inhibition zones compared with others were very effective against *Candida* in broth dilution assays,[10] which do not rely on diffusion. Juniper oil exhibited a ZOI of 5.6 mm in the disc diffusion assay, whereas castor oil showed 7.8 mm. However, juniper oil (2%) performed better than castor oil (3%) in the MFC assay. Fungicidal activity is considered as a desirable quality for antifungal agents, since it can totally eliminate the fungus from tissues. *C. albicans* showed differing sensitivity to the 30 plant oils tested. The majority of the oils was effective and showed anti-*Candida* activity at very low concentrations (Table). Interference of peppermint oil with iron uptake has been reported, which suggests an interaction with normal metabolic activities.[13]

Fluconazole is known to be very effective against human pathogenic fungi and is used as the drug of choice to treat systemic fungal infections and, despite its severe side effects, may require prolonged use.[14–16] It is encouraging to note that the majority of oils used in this study were fungicidal at low concentrations. Until now not much information was available about the mode of action of natural products that inhibit *Candida* growth. More fluconazole/azole-resistant strains need to be included in future studies. Plant oils could find use as anti-*Candida* agents against azole-resistant strains. Most of the oils used in this study have a long history of use in food, confectionery and as components of perfume.[1] However, before they are considered for use as topical preparations, a careful exploration of their undesirable effects needs to be undertaken.

In summary, the results presented in this paper clearly demonstrate the antifungal potential of selected plant oils. Eucalyptus, peppermint, clove and ginger grass oils act as fungistatic, fungicidal and anti-biofilm agents against *C. albicans*. Eucalyptus and peppermint oils may be used as anti-biofilm agents at very low concentrations. These results not only encourage further examination of the efficacy of plant oils against other forms of systemic and superficial fungal infections, but also the exploration of their broad spectrum effects against other pathogenic manifestations, including malignancies, in the coming years.

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