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Antifungal Activity of Essential Oils against Fluconazole Resistant Fungi

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A B S T R A C T

Pathogenic fungi like Candida albicans, Candida tropicalis and Trichophyton mentagrophytes are commonly encountered strains associated with a wide range of conditions including scalp infection, oral thrush, skin infection, and vaginal thrush. Unlike bacterial pathogens, fungal pathogens are difficult to control. Fluconazole is a commonly administered antifungal drug. The medical fraternity has been reporting an alarming increase in the development of resistance observed amongst fungal strains to Fluconazole. The present study involves screening of essential oils for their antifungal activity against Fluconazole resistant fungi.

Essential oils of Black pepper, Cardamom, Cumin, Boswellia and Patcholi were selected for the study. The results indicated that all the oils inhibited fungal strains in varying degrees of dilutions. Essential oil of Boswellia was found to be the most effective in antifungal activity against Candida tropicalis and essential oil of Cardamom against Trichophyton mentagrophytes. To assess the effect of combination of essential oils with Fluconazole, synergistic action was also studied. The results indicated that essential oil of Boswellia and Fluconazole in combination acted as the most powerful antifungal agent against Candida tropicalis even at 1:10 dilution and 100μg/ml respectively.

These results lead us to believe that active components present in essential oils should be a focus area of future in vivo research, especially in conjunction with existing antifungal drugs. The molecular mechanisms, mode of action, stability, toxicity, and efficacy of the active components isolated from essential oils need to be further studied and evaluated.

Keywords: Essential oils, antifungal drug, drug resistance, synergistic action.

Introduction

Over the last few years, the indiscriminate use of antibiotics has led to the problem of multi-drug resistance. This situation has forced researchers to search for new antimicrobial substances from medicinal plants. The curative properties of aromatic and medicinal plants have been recognized since ancient times [1]. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants because plant-derived drugs have been reported to be safe and without side-effects [2].

Down the ages, essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases. The World Health Organization (WHO) has noted that the majority of the world's

population depends on traditional medicine for primary healthcare. The modification of antibiotic activity is done by compounds that enhance the activity of the antibiotic, and plants are a rich source of these compounds [3].

Essential oils (also called volatile oils) are natural, complex, aromatic, oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots) and composed mainly of terpenes, in addition to some other non-terpene components. They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production. They have been shown to possess antibacterial, antifungal, antiviral, insecticidal, and anti-oxidant properties [4][5]. The hydrophobicity of essential oils enables them to partition in the lipids of the cell membrane and mitochondria, rendering them permeable and leading to leakage of cell contents [6].

Concomitant with its widespread use, there have been increasing reports of Fluconazole resistance. While there is a perception that this problem is growing in frequency, its true reason is not known, nor is an optimum strategy for its prevention apparent [7]. Alternative therapies need to be developed to suppress the emergence of antifungal resistance. Conventional medicine applies the "silver bullet" method, where single target therapy is employed. The recent trend has been the "herbal shotgun" method like Ayurveda, where multitargeted approach of herbals and drugs is used. This can be achieved by the use of combinations of existing agents or the development of new, safer and effective agents primarily from plant sources which can exhibit synergy with drugs [8].

The aim of this study was to test five essential oils and to study the synergistic action between Fluconazole and essential oils for their antimicrobial activity against Fluconazole-resistant fungi.

Materials and methods

Test Organisms

The fungal strains used in the study were Candida albicans, Candida tropicalis and Trichophyton mentagrophytes. They were obtained from Brahmakumari Hospital, Mumbai, India. The cultures were maintained on Sabouraud's agar slants containing 50μg/ml of Fluconazole. Inocula were prepared by diluting overnight cultures in saline. Fresh subcultures were used for each experiment.

Essential oils

Essential oils of Black pepper, Cardamom, Cumin, Boswellia and Patcholi were used for the study. They were obtained from Konark Herbals and Healthcare, Mumbai, India.

Antifungal Activity of Essential oils

The antifungal activity of essential oils and the dilutions of essential oils prepared in propylene glycol were tested by the agar well diffusion method. The suspension of fungal strains in saline was swabbed on sterile Sabouraud's agar plates. Wells were punched in the Sabouraud's agar plate with the help of sterile cork borer of 9mm diameter. 100μl of different oils was then dispensed inside the wells with the help of a micropipette. The plates were then kept in the refrigerator for two hours and then incubated at 37ºC for 24 to 48 hours. The plate with T.mentagrophytes was incubated at room temperature for 3 days. After incubation, the plates were observed for the zone formation and the diameter (mm) of the zone was measured and tabulated (Tables 1 and 2).

Synergistic Activity

For synergistic activity between Fluconazole and essential oils, sterile strips of 5cmx1cm were used. The strip containing 100μg/ml of Fluconazole and the strip containing 100μl of 1:10 diluted essential oil were then placed perpendicular to each other (making a 'plus sign') aseptically on a sterile Sabouraud's agar plate swabbed with the fungal strain. This procedure was done for all the essential oils and Fluconazole against all the fungal strains. The plates were then incubated at 37ºC for 24 to 48 hours. The plates with T.mentagrophytes were incubated at room temperature for 3 days. After incubation the results were recorded and tabulated (Table 3)

Results & Discussion

The antifungal activity of five essential oils and their dilutions against the three fungal cultures are shown in Tables 1 and 2. Cardamom, Black pepper, Cumin and Boswellia inhibited all the three fungal cultures even at 1:10 dilution. Patcholi oil was ineffective as it did not inhibit any of the fungal cultures tested.

Table 1: Antifungal activity of essential oils.

	Zone of inhibition in mm				
Fungal cultures	**Cardamom oil**	**Black pepper oil**	**Cumin oil**	**Patcholi oil**	**Boswellia oil**
Candida albicans	24	16	20	No inhibition	28
Candida tropicalis	18	15	17	No inhibition	26
Trichophyton mentagrophytes	28	36	22	No inhibition	25

Table 2:

(1) Antifungal activity of dilutions of Cardamom oil.

Name of the culture	Zone of inhibition in mm				
	1:2	**1:4**	**1:6**	**1:8**	**1:10**
Candida albicans	21	19	17	15	13
Candida tropicalis	15	14	13	12	10
Trichophyton mentagrophytes	26	23	20	17	15

(2) Antifungal activity of dilutions of Black pepper oil.

Name of the culture	Zone of inhibition in mm				
	1:2	**1:4**	**1:6**	**1:8**	**1:10**
Candida albicans	14	13	12	11	9
Candida tropicalis	12	11	10	9	8
Trichophyton mentagrophytes	34	31	28	26	23

(3) Antifungal activity of dilutions of Cumin oil.

Name of the culture	Zone of inhibition in mm				
	1:2	**1:4**	**1:6**	**1:8**	**1:10**
Candida albicans	18	16	14	12	10
Candida tropicalis	15	13	11	9	6
Trichophyton mentagrophytes	20	17	15	13	10

(4) Antifungal activity of dilutions of Boswellia oil.

Name of the culture	Zone of inhibition in mm				
	1:2	1:4	1:6	1:8	1:10
Candida albicans	24	23	21	20.5	19
Candida tropicalis	24.5	22	19	17	15
Trichophyton mentagrophytes	23	19	15	14	12

The results of synergy (Table 3) indicated that the combination of Fluconazole and Cardamom oil exhibited good antifungal activity against C.albicans. However, the combination of Fluconazole and Boswellia oil exhibited the most powerful antifungal activity against C.tropicalis even at 1:10 dilution. No synergistic action was observed for Fluconazole and any of the essential oils in combination against T.mentagrophytes.

Table 3: Results of Synergistic action.

Name of the culture	Agent(s) used	Zone of Inhibition (mm)
Candida albicans	Fluconazole	7
	Cardamom oil	5
	Fluconazole + Cardamom oil	14
Candida tropicalis	Fluconazole	3
	Boswellia oil	4
	Fluconazole + Boswellia oil	12

Essential oils have been known to possess antimicrobial activity by their action through the disruption of the cell membrane [9]. It is important to investigate those plants that have been used in traditional medicine as potential sources of novel antimicrobial compounds [10].

In general, the comparison of results involving in vitro studies of plant extracts is problematic. First, the composition of plant oils and extracts is known to vary according to local climatic and environmental conditions. Secondly, the method used to assess antimicrobial activity and the choice of test organism(s), varies between publications [11][12]. For the abovementioned reasons, in vivo studies may be required to confirm the validity of the results obtained.

Conclusion

In order to select a compound that could act in synergism with a drug, it is necessary to understand the complete molecular mechanism of the drug action in the presence and absence of the natural compound. The problems that still need to be addressed are stability, selectivity and bioavailability of these natural products, and any adverse herb-drug interaction [8].

The applications of the above studies of these compounds in the in vivo systems or in clinical studies require pharmacokinetic and pharmacodynamic parameters. The interaction of drugs leading to either synergism or antagonism is not indicative of their pharmacodynamic efficacy [13]. In summary, this study confirms that many essential oils possess in vitro antifungal activity. However, if essential oils are to be used for medicinal purpose, issues of safety and toxicity will need to be addressed.

Authors' contributions

AR conceptualized the research, and conducted the experiment and analysis. SD and DK helped in study design, and assisted AR with the drafting of the manuscript. All authors have read and approve of the final manuscript.

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References

1. Lixandru BE, Dracea NO, Dragomirescu CC, Dragulescu EC, Coldea IL, Anton L, Dobre E, Rovinaru C, Codita I. Antimicrobial activity of plant essential oils against bacterial and fungal species involved in food poisoining and/or food decay. Roum Arch Microbiol Immunol. 2010;69(4):224-230. www.ncbi.nlm.nih.gov/pubmed/21462837
2. Bansod S, Rai M. Antifungal Activity of Essential Oils from Indian Medicinal Plants Against Human Pathogenic Aspergillus fumigatus and A. niger. World J of Medical Sciences. 2008;3(2):81-88. http://idosi.org/wjms/3(2)08/7.pdf
3. Gibbons S. Plants as a source of bacterial resistance modulators and anti-infective agents. Phytochem Rev. 2005;4:63-78. http://210.27.80.89/2005/zhihua/file/2005pdf/51Plants%20as%20a%20source%20of%20bacterial%20resistance%20modulators.pdf
4. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine. 2006;6:39. http://www.biomedcentral.com/1472-6882/6/39
5. Coutinho HDM, Matias EFF, Santos KKA, Tintino SR, Souza CES, Guedes GMM, Santos FAD, Costa JGM, Falcao-Silva VS, Siqueira-Junior JP. Enhancement of the norfloxacin antibiotic activity by gaseous Contact with the essential oil of Croton zehntneri. Pharmacognosy. 2010;2(4):362-364. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3019373/
6. Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review. Int.J. Food Microbiol. 2004;94(3):223-253. http://www.ncbi.nlm.nih.gov/pubmed/15246235
7. Rex JH, Rinaldi MG, Pfaller MA. Resistance of Candida Species to Fluconazole. Antimicrob. Agents Chemother. 1995;39(1):1-8. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC162475/
8. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008;15:639-652. http://www.sciencedirect.com/science/article/pii/S0944711308001104
9. Shiota S, Shimizu M, Mizushima M, Ito H, Hatano T, Yoshida T, Tsuchiya T. Restoration of effectiveness of beta-lactams on methicillin resistant Staphylococcus aureus by tellimagrandin I from rose red. FEMS Microbiol. Lett. 2000;185:135-138. http://www.ncbi.nlm.nih.gov/pubmed/10754237
10. Mitscher LA, Drake S, Gollapudi SR, Okwute SK. A modern Look at folkloric use of anti-infective agents. J of Natural Products. 1987;50:1025-1040. http://www.ncbi.nlm.nih.gov/pubmed/3443855
11. Janssen AM, Scheffer JJC, Baerheim Svendsen A. Antimicrobial activity of essential oils: a 1976-86 literature review. Aspects of the test methods. Planta Medica. 1987;53:395-398. http://www.ncbi.nlm.nih.gov/pubmed/3324126
12. Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M. Antimicrobial activity of mint essential oils. J of Agriculture and Food Chemistry. 1995;43:2384-2388. http://pubs.acs.org/doi/abs/10.1021/jf00057a013
13. Den Hollander JG, Mouton JW, Verbrugh HA. Use of pharmacodynamics parameters to predict efficacy of combination therapy by using fractional inhibitory concentration kinetics. Antimicrob. Agents Chemother. 1998;42:744-748. http://aac.asm.org/content/42/4/744