ARTICLE

Antifungal Properties of Medicinal Plant Extracts against *Candida albicans*

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Abstract

Medicinal plant extracts prepared with selected aqueous concentrations from three species namely AzadirachtaindicaA.Juss., SimaroubaamaraAubl. andOcimumtenuiflorumL. were tested onto the fungus Candida albicans using the standard Agar well Diffusion Assay. Plant extracts showed relatively strong antifungal action against the fungus Candida albicans. In the present study, strong antibacterial activity was found at maximum concentration of the sample, ie. 1000 µg/ml. Azadirachta indica showed relatively strong antifungal activity for Candida albicans in all sample concentrations such as 250 µg/ml, 500µg/ml and 1000 µg/ml, when compared to the other two species. All the medicinal plant extracts showed satisfactory level of inhibitory action against growth of the fungus. These results indicate that extracts of plants can offer significant potential for the development of novel antimicrobial therapies

Key words : *Antifungal, Candida, Extract, Medicinal*

Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. The increasing acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to available antibiotics haveled to the investigation of the antimicrobial activity of medicinal plants (Bisignano*et al*.1996;Lis-Balchin and Deans 1996). Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries highlight the importance of a systematic study of medicinal plants in order to find medicinally active phytocompounds.

The control of microbial infection has been remarkably effective since the discovery of antibacterial drugs. However, some of the pathogens rapidly become resistant to many of the pioneer effective drugs. The development of drug resistance as well as undesirable side effects of certain antibiotics (WHO, 2003) have led to the research for new antibacterial as well as antifungal agents, particularly from medicinal plants.

Candida albicans is an opportunistic pathogenic yeast (Gow, 2017) that is a common member of the human gut flora. It does not proliferate outside the human body (Odds,1988). It is detected in the gastrointestinal tract and mouth in 40–60% of healthy adults (Kerawala and Newlands, 2010). It is one of the few species of the genus *Candida* that causes the human infection candidiasis, which results from an overgrowth of the fungus (Martins *etal*. 2014). *Candidiasis* is for example often observed in HIV-infected patients (Calderoneand Clansy, 2012). A mortality rate of 40% has been reported for patients with systemic candidiasis due to *C. albicans* (Singhand Chakrabarti, 2017).

The aim of this study was to investigate the antifungal activity of extracts from medicinal plants used in folk medicine, and a comparative study was made on the antifungal properties of extracts from three medicinal plants *viz.Azadirachtaindica*, *Simaroubaamara* and *Ocimumtenuiflorum* on *Candida albicans*.

Materialsand Methods

Fresh leaves of *Azadirachtaindica*, *Simaroubaamara* and *Ocimumtenui florum* were collected from SreeNarayana College campus, Chempazhanthy, Thiruvanathapuram, Kerala, South India and nearby areasfor preparing the three plant extracts. The freshly collected leaf samples were thoroughly washed in tap water, followed by sterile distilled water. The plant material of each sample was dried in an oven at 50°C for 48 h, followed by grinding into a fine powder. The powdered material was stored in air tight jars in refrigerator at 4°C. 10 grams of plant powder was extracted with 90% ethanol for 72 hours and the extract

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was recovered by filtration using double layered muslin cloth. The extract was air dried and 100 mg of dried extract was dissolved in 1 ml of Dimethyl sulfoxide (DMSO).

Agar-Well Diffusion Method

Principle

The antifungal activity was determined by Agar well diffusion method in order to assess the biological significance and ability of the sample. The antifungals present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Materials Required

1. Potato Dextrose Agar Medium (1L): The medium was prepared by dissolving 39 g of the commercially available Potato Dextrose Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30ml/ plate) while still molten.
2. Clotrimazole (standard antifungal agent, concentration: 10mg / ml)
3. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5% - *Candida albicans* (ATCC 10231)

Procedure

Potato Dextrose agar plates were prepared and overnight grown species of fungus, *Candida albicans* were swabbed. Wells of approximately 10mm were bored in the agar plate using a well cutter and samples of different concentrations such as 250µg/ml, 500µg/ml and 1000µg/ml were added. The inoculated agar plates were then incubatedovernight at room temperature. After incubation the zone of inhibition was measured and compared with that of the standard antimycotic (Clotrimazole) (NCCLS, 1993).

Results and Discussion

The extracts of the medicinal plants tested were effective antimicrobial agents against a group of microorganisms. All the threeplant samples were sensitive to the fungus (Table 1; Plate-2).In the present study, strong antifungal activity was found at maximum concentration of the sample, *ie*., 1000 µg/ml. *Azadirachtaindica*showed relatively strong antifungal activity for *Candida albicans*in all sample concentrations such as 250 µg/ml,500 µg/ml and 1000 µg/ml, withthe zone of inhibition being 16mm. But in the *Ocimumtenuiflorum*sample, the maximum antifungal activity was expressed at 1000 µg/ml concentration and the zone of inhibition was measured as 12 mm. Overall, medicinal plant extracts from the plant,*Azadirachtaindica*showed satisfactory level of inhibitory action against growth of *Candida albicans.* The plant sample, *Simaroubaamara*expressed at 1000 µg/ml concentration, the zone of inhibition was measured as 11 mm only.

Table1 :Antifungal Activity of Three Plant Extracts against *Candida albicans*

Sl No	Sample	Mode of Identification	Concentration (µg/ml)			
			Control (Clotrimazole)	250	500	1000
1.	*Azadirachtaindica*	Zone of Inhibition (mm)	28	Nil	10	16
2.	*Simaroubaamara*		28	Nil	10	11
3.	*Ocimumtenuiflorum*		28	Nil	10	12

The type and level of biological activity exhibited by any plant material depends on many factors including the plant part, geographicalsource, soilconditions, harvest time, moisturecontent, dryingmethod, and storage conditions.This has also been stressed by earlier workers who opined that the age and freshness of the plant material used,level of contamination of the sourcematerial collected fromthefield, adulteration of the test material, abiotic components or factor saffecting the extraction procedure, errors in the preparation of the extract and the dosage administered are all factors which contribute to such differences in th eantimicrobial efficacy of different plantextracts(Calixto,2000; OkigboandIgwe, 2007). The extraction procedure may be accompanied by several errors. For instance, the relatively high temperature that may be generated during tissue grinding can denature chemical constituents in the extraction solvent. The durationof extraction and temperature can affect the level and composition of secondary metabolites in theplant extract. The variation in the composition of active compounds in different plant types may necessitate the requirement

of different concentrations of ethanol to achievea maximum reco very of bioactive components. Further, no standardized extraction protocol has been developed for the preparation of her balextracts. However, 20-95%of ethanol-water mixture is frequently used by the herbal medicine industry to prepare ethanolic extracts (Ganora,2008).

According to the World Health Organization, infectiousdiseases are a significant cause of worldwide morbidity and mortality, accounting for approximately 50% of all deaths in tropicalcountries (WHO,2003). In the US, disease hospitalization rates have increased over time and are associated with substantial morbidity, mortality, andeconomicconsequences.Additionally, antimicrobial resistance to antibiotics is emerging as a serious health issue and alternatives to treat infectious diseases in the future need to be developed(Abascal and Yarnell,2002).

A number of studies have voiced the necessity of developing alternative antimicrobial drugs (Poole, 2002; Sibanda and Okoh, 2007). Plant antimicrobials would appear to be an excellent choice (Mahady, 2005).The present study provides data on the importance of selection of an appropriate solvent concentration and indicates that extracts of plants can offer significant potential for the development of novel antibacterialtherapies.

Conclusions

Plant extracts prepared with selected aqueous concentrations from three medicinally important species namely, *Azadirachtaindica, Simaroubaamara* and *Ocimumtenuiflorum* were tested onto the fungus *Candida albicans*using the standard Agar well Diffusion Assay.All the three medicinal plant extracts showed satisfactory level of inhibitory action against the growth of the fungus. Strong anti bacterial activity was found at maximum concentration of the sample, ie., 1000 µg/ml. *Azadirachtaindica* showed relatively strong antifungal activity for *Candidaalbicans* in all sample concentrations such as 250µg/ml,500 µg/ml and 1000 µg/ml, with the largest zone of inhibition (16mm), while the inhibition zone measured only up to 12 mm and 11mm for *Ocimumtenuiflorum* and *Simaroubaamara* respectively. Theseresults indicate that extracts of plants can offer significant potential forthe development of novel antimicrobialtherapies. The study also highlights the need to create awareness among people about the significance of herbal plants.

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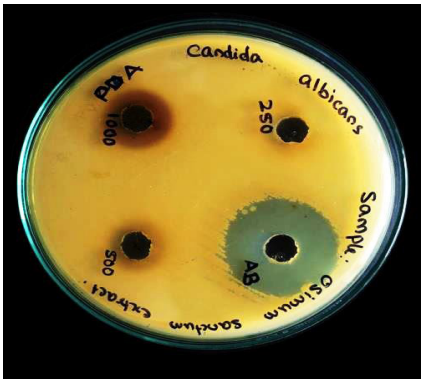
Plate-1

Azadirachta indica

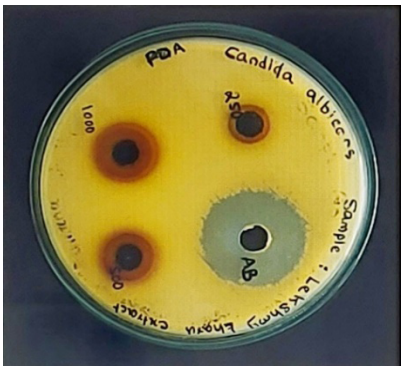
Simarouba amara

Ocimum tenuiflorum

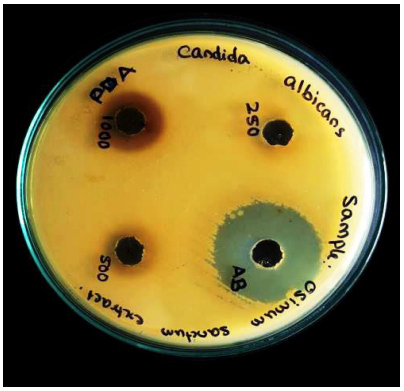
Plate-2



Sample- ***Azadirachta indica***



Sample- ***Simarouba amara***



Sample- ***Ocimum tenuiflorum***

Note: Concentration of stock 10mg/ml DMSO