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Original Research Article

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Antifungal Activity of Weed Extracts on *Candida Albicans* : An *In-vitro* Study

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Abstract

The genus *Candida* is caused morbidity and mortality in human beings. The virulence factors of the *Candida albicans* have the great role in the pseudohyphae formation by attached with epithelial cells and endothelial cells. The aim of the study was to evaluate the antifungal activity of weed extracts used in *traditional* herbal medicine. The weeds were selected on the basis of their reported ethno botanical uses. Water and alcoholic weed extracts were screened *in vitro* for their antifungal activity against fungus *C. albicans*. 50μl concentration of minimal Inhibitory Concentration (MIC) of *Mimosa pudica* extract in alcohol and 70μl concentration of MIC of *Oxalis corniculata* extract shows in alcohol. We conclude from this that these extracts exhibit amazing fungicidal properties that support their traditional uses. The presence of phytochemicals in the extracts including, tannins, alkaloids, steroids, glycosides, triterpenoids, flavonoids, phenolic compounds and organic acids like, Malic acid, tartaric acid and citric acid may be responsible for these activities. The acetone extracts of plant are more efficient as compared to the water extract.

Keywords: *Candida Albicans*, weed, *Oxalis corniculata*, *Mimosa pudica*, anti-fungal

Introduction

Candida is a genus of yeasts and is the most common cause of fungal infections worldwide [1]. Candida is the most common fungal pathogen of humans and the fourth leading pathogen in nosocomial bloodstream infections [1]. The most common superficial candidiases are oral candidiasis and Candida associated denture stomatitis [2-4]. Candida-associated denture stomatitis could affect up to 70% of denture wearers [5]. *Candida albicans* is the most commonly isolated species, and can cause infections (candidiasis or thrush) in humans and other animals. In winemaking, some species of *Candida* can potentially spoil wines. *C. albicans* are most important species and it is responsible for oral thrush, candidiasis, candiduria and *Candidemia* frequently seen in patients. The *Candida* species found as normal flora in human beings. Common sites are skin, gastrointestinal tract and female genital tract particularly higher in vagina during pregnancy. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. The effects of plant extracts on bacteria and fungi have been studied by a very large number of researchers in different parts of the world [6,7]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. Plant extracts have played significant role in the inhibition of seed-borne pathogens and in the improvement of seed quality and field emergence of plant seeds [8]. Contrary to the synthetic drugs, antifungal of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. If this medicinal or antifungal property resides in a weed that will be an added advantage. The present investigation istherefore, undertaken to test the efficacy of some of the common weed extracts against *Candida albicans in vitro*.

Materials and Method

Plant material

Oxalis corniculata (Family: Oxalidaceae) and *Mimosa pudica* (Family: Fabaceae) weed materials were collected in local areas of Bangalore, Karnataka, India. And authenticated by Dr. T. Sridhar Bairy by comparison with the standard specimens deposited at the Department of Drava Guna, SDM College of Ayurveda, Udupi. Voucher specimen is kept at the Acharya and B M Reddy College of pharmacy, Bangalore, Karnataka, India.

Preparation of extracts

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Both plant materials (*Oxalis corniculata* and *Mimosa pudica*) were collected from local areas of Bangalore, Karnataka. India, and then dried in shade. After complete dry, fine powder was made by electric grinder. 5g powder sample was added in 50 ml alcohol and distilled water then kept it for shaking in orbital shaker for 72h at room temperature. After incubation the extracts were filtered with muslin cloth followed by what man filter paper [9]. The extracts were added into clean petri plate for evaporation then allowed for evaporation. (The used petri plates were reweighted). After evaporation, the plates were weighted. Residual concentrates were dissolved in 5 ml of DMSO. The extracts were collected in screw capped bottles. The extracts were used for antifungal activity, MIC test and phytochemical test. The extracts were stored at 20 C for experimental use. Bio efficacy of the extract was checked *in vitro* by well in agar diffusion method [10].

Organisms

The standard strain used for the study is *Candida albicans* (ATCC 66027). All isolated were identified by spore germination test, production of chalmydoconida on corn meal agar [11].
These isolates were grown on Sabourauds dextrose agar (SDA) (HIMEDIA Laboratories Pvt. Ltd; Mumbai, India) at 4 C.

Activation of fungi (*Candida albicans*)

Loopful fungal spores were streaked on potato dextrose agar (Hi-media) plates and incubated at 37 C for 2-3 days. All fungus plates were maintained at 4 c in refrigerator for further use.

Zone of Inhibition

For determination of zone of inhibition, basically three methods are used. One of them is a well diffusion method which we have used.

Media used for the Study

The Sabouraud's dextrose agar (SDA) (Hi Medic Laboratories Pvt. Ltd; Mumbai, India) was used for the study.

Preparation of potato dextrose agar medium (PDA agar medium)

Preparation of PDA includes the following steps

PDA agar medium was prepared from commercially available dehydrated base according to the manufacturer instructions.
Immediately after autoclaving, allowed to cool in 45 to 50 C water bath. The freshly prepared and cooled medium was poured into the glass or plastic flat bottomed Petri dishes till the level, horizontal surface to give uniform depth. The PDA agar medium should be allowed to cool at room temperature and until the use plates were stored in a refrigerator. Plates should be used within 7 days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of agar. Representative samples of each batch of plates were examined for sterility by incubating at 30-35 C for 24hours.

Preparation of well

The wells were made using cork borer on N-agar plate. The borer was dipped into the alcohol for sterilization and then was used to make wells. Plates were used for the zone of inhibition test.

Procedure for performing the well diffusion method Inoculums preparation

Three to five well-isolated colonies of the fungus were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4-5 ml of PDA broth medium. The broth culture is incubated at 350 C until it achieves turbidity 1-2 x 108 CFU/ml. The turbidity of actively growing broth culture was adjusted with sterile saline.

Inoculum of test plates

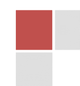
Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension, loopful of suspension inoculates into flask contains Agar. Mix it well and pour it into plate and rotate the plate for even distribution. On the dry PDA agar plate loopful suspension evenly spreaded by spreader.

Note

Extremes in inoculums density must be avoided. Never use undiluted overnight broth culture or other unstandardized inoculums for streaking plates.

Inoculums of plant extract into the well

In the plate, wells were made for the inoculation of weed extract. Minimum four wells were made in one plate.
Using micropipette, 30μl of antifungal drug was added and extracts into respective wells.
The plates were first placed at 4 C for 30 min in order to diffusion of extract and antifungal drug.
Then plates were incubated at 37 C for 24 hours at room temperature.



The diameter of the inhibition zones were measured in millimeter at the end of the incubation time.

Determination of Minimal Inhibitory Concentration (MIC)

Dilution susceptibility testing method was used to determine the minimal concentration of antifungal to inhibit or kill thefungus. This was achieved by dilution of antifungal to inhibit or kill the fungus and was achieved by dilution of antifungal in either agar or broth media (PDA).

Procedure for performing the minimum inhibitory concentration Test inoculums

Preparation

Inoculums preparation was performed as discuss earlier in well diffusion method.

Procedure

Different concentrations of weed extracts in (10μl, 20μl.FFup to 100μl) to the tube to respective tubes were added.
From the inoculums 10μl of each culture was inoculated separately in each set so that final concentration of fungus in tubes became 106 cells/ml. This procedure was performed for all the positive extracts antifungal activity which were obtained by primary screening.
Then all sets of tubes of dilution broth were incubated at 37 C for 24 hours in incubator.
All sets of tubes were observed for determination of MIC to the susceptible fungus were tested and note down the results.

Results

Screening and evaluation of antifungal activity

The screening and evaluation of antifungal activity was carried out by agar well diffusion method and determination of MIC values, which was carried out by using different concentrations of weed extracts table 1. The test fungus was *Candida albicans.*

Table-1: Concentration of each extract used to check antifungal activity

Sl No	Plant weed name	Water extract (Conc. mg/ml)	Alcoholic extract (Conc. mg/ml)
1	*Oxalis corniculata*	16.7	19.3
2	*Mimosa pudica*	12.3	14.2

Results of diameter of inhibition zone (DIZ value)

The measured DIZ of various extracts of weed with different solvents against *C. albicans*are shown in table 2.

Table-2: Results of diameter of inhibition zone

Sl No	Plant weed name	Diameter of inhibition zone (DIZ) (mm)	
		Water extract	Alcoholic extract
1	*Oxalis corniculata*	-	24.23
2	*Mimosa pudica*	12.3	16.2

Results of determination of MIC value

After evaluating the DIZ values extracts (*Oxalis corniculata* and *Mimosa pudica* extract *prepared by alcohol)* were taken which shows higher antifungal activity for MIC test by taking a different concentration (Table 3). The test fungus was inoculated in different concentration of weed extracts i.e.10μl, 20μl, FF.100μl.

Table 3: Results of MIC for alcoholic extracts

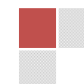
Sl No	Extracts	Different volume of plant extracts (μl)									
		10	20	30	40	50	60	70	80	90	100
1	*Oxalis corniculata*	+	+	+	+	+	+	+	-	-	-
2	*Mimosa pudica*	+	+	+	+	+	-	-	-	-	-

The growth of *C. albicans* (ATCC 66027) *in Mimosa pudica* alcoholic extract was seen below 50μl concentration. So, 40μl can be said MIC of *M. pudica* extract. The growth of *C. albicans* in *Oxalis corniculata* alcoholic extract was seen below 70μl concentration. So, 70μl can be said MIC of *O. corniculata* extract.

Phytochemical Test

The results of qualitative screening of phytochemical components in revealed the presence of tannins, alkaloids, steroids, glycosides, triterepenes, flavonoids, phenolic compounds and organic acids like Malic acid, tartaric acid, citric acid which are responsible for anti-fungal activity in *Oxalis corniculata* whereas tannins, saponins, alkaloids, flavonoids, steroids, proteins present in *Mimosa Pudica*

Discussion



Candida albicans is the most common *Candida* species residing in the oral cavity in both health and disease and is the agent of most oral *Candida* infections. Several effective antifungal agents were available for the management of candidiasis. But isolates may exhibit intrinsic or secondary resistance to the drug during therapy. So the use of natural products as alternative agents for the control of fungal diseases is considered as an interesting alternative to synthetic fungicides. *Oxalis corniculata* and *Mimosa Pudica* weed extracts shows antifungal activity. *C. albicans* was strongly inhibited by the alcoholic extract of Indian sorrel, followed by alcoholic extract of *Mimosa Pudica* when compared to aqueous extract. This suggests that weed extracts can be used to inhibit the growth of *C. albicans* and thus they can be implicated in the prevention and treatment of oral candidal infections. The efficacy of weeds and their extracts was due to the presence of several primary and/or secondary metabolites such as tannins, alkaloids, steroid, glycosides, triterepenes, flavonoids, phenolic compounds and organic acids like Malic acid, tartaric acid, citric acid and complex mixtures. Although phytochemicals (plant derived metabolites) are antimicrobial in nature but they also produce other biological activities in the oral cavity like induction of immunity, which indirectly reduces the risk of oral diseases.

Summary and Conclusion

These weeds *Oxalis corniculata* and *Mimosa Pudica* were used for antifungal screening against *Candida albicans*. The weed materials were extracted with alcohol. For the diameter of inhibition zone, minimum inhibitory concentration, were determined by well diffusion method and potato dextrose broth dilutions. The phytochemical analysis was made to determine active inhibitors present in extracts including, steroid, triterpenes, alkaloids, tannins, flavonoids, lactones, diterpines, glycosides, saponins. The result obtained in this study clearly demonstrates broad spectrum antifungal activity of *Oxalis corniculata* and *Mimosa Pudica* extract against *Candida albicans.* The presence of phytoconstituents in the extracts including, (tannins alkaloids, steroid, glycosides, triterepenes, flavonoids, phenolic compounds and some organic acids) may be responsible for these activities. Alcoholic extracts of weed were more efficient as compared to the aqueous extracts.

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