Int J Pharm Bio Sci 2015 Oct; 6(4): (P) 701 - 706

Research Article **Pharmacognosy**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

ANTIFUNGAL ACTIVITY OF TWO MEDICINAL PLANTS AGAINST FUNGUS *CANDIDA ALBICANS*

MAYURI C. RATHOD[1], NAMRATA DAS[2] AND D. A. DHALE[3]*

[1,2]Biotechnology Course, Veer Narmad South Gujarat University, Surat – 395007, (Gujarat)
[3]Department of Botany, SSVPS's, L.K.Dr.P.R.Ghogrey , Science College, Dhule-424005 (MS)

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 extracts of two plant species used in *traditional* herbal medicine. The plants were selected on the basis of their reported ethnobotanical uses. Aqueous and acetone extracts of two plant species were screened *in vitro* for their antifungal activity against fungus *C. albicans*. 40µl concentration of Minimal Inhibitory Concentration (MIC) of Sandalwood extract in acetone and 60µl concentration of MIC of Arjun barks extract shows in acetone. We conclude from this that these extracts exhibit amazing fungicidal properties that support their traditional uses. The presence of phyto-compound in the extracts including, steroid, triterpenes, alkaloids, tannin, flavnoids, lactones, diterpines, giycosides, sapponins may be responsible for these activities. The acetone extracts of plant are more efficient as compared to the water extract.

KEY WORDS: ***Candida albicans,*** **Ethnobotany, Fungicidal Properties, Traditional Medicine**

D. A. DHALE
Department of Botany, SSVPS's, Late K.Dr.P.R.Ghogrey Science College, Dhule-424005 (MS), India

***Corresponding author**

INTRODUCTION

Candida is a genus of yeasts and is the most common cause of fungal infections worldwide[1]. Many species are harmless commensals or endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease[2]. *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[3]. *C. albicans* are most important species and it is responsible for oral thrush, candidiasis, candiduria and Candidemia frequently seen in patients and it is also responsible to cause vulvovaginitis in girls at the pubeteric age group. The incidence of *Candida* species is significantly increases over the past two decades and non-albicans *Candida* (NAC) continue to replace *C. albicans* at most of the clinical sites i.e. blood stream infections. 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[4]. Medicinal plants represent a rich source of antimicrobial agents[5]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. Plants generally produce many secondary metabolites which constitute an important source of micro biocides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional 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[6,7,8]. Much work has been done on ethno medicinal plants in India. Interest in a large number of traditional natural products has increased. Exploitation of plant metabolites in crop protection and prevention of biodeterioration caused by fungi appear to be promised. In view of these, the author screened some extracts against vegetable pathogenic fungi and the data has been presented in this paper. Hence, there is a great demand for novel antifungal belonging to a wide range of structural classes, selectively acting on new targets with fewer side effects. One approach might be the testing of plants traditionally used for their antifungal activities as potential sources for drug development. Medicinal plants were not only important to the millions of people for whom traditional medicine is the only opportunity for health care and to those who use plants for various purposes in their daily lives, but also as a source of new pharmaceuticals. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the matched less availability of chemical diversity.

MATERIALS AND METHODS

Plant material: Different plant tissues from plant species used in traditional medicine (Table 1) were collected in 2014 in their natural habitat in around Surat City (Gujarat). The collected plants were identified at the Department of Botany, Veer Narmad South Gujarat University, Surat (Gujarat)[9]. The stem of Sandalwood and stem bark of Arjun is then shade dried and grind in electric mixer grinder. The powder material was kept in air tight glass bottles. This stock powder was used for further extraction.

Table 1
List of plant species

Sr. No.	Plant Name	Common Name	Family	Part Used
1	*Santalum album* Linn.	Sandalwood	Santalaceae	Wood
2	*Terminalia arjuna* (Roxb.) Wight & Arn.	Arjun	Combretaceae	Stem Bark

Preparation of extracts

Both plant materials were collected, and then dried in shade. After complete dry, fine powder was made by electric grinder. 5g powder sample was added in 50 ml acetone 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 Whatman filter paper[10]. The extracts were added into clean petriplate for evaporation then allowed for evaporation. (The used petriplates were pre-weighted). 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. Bioefficacy of the extract was checked *in vitro* by well in agar diffusion method[11].

Organisms

Candida albicans were obtained from the Department of Biotechnology, Veer Narmad South Gujarat University, Surat (Gujarat). All isolates were identified by germ-tube test[12], spore germination test[13], production of chlamydoconidia on corn meal agar[14]. These isolates were maintained on Sabouraud's dextrose agar SDA (HIMEDIA Laboratories, Mumbai-India) at 4ºC.

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.

A. Preparation of potato dextrose agar medium (PDA agar medium)

Preparation of PDA includes the following steps

i. PDA agar medium was prepared from commercially available dehydrated base according to the manufacturer instructions.
ii. Immediately after autoclaving, allowed to cool in 45 to 50°c water bath.
iii. 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.
iv. The PDA agar medium should be allowed to cool at room temperature and until the use plates were stored in a refrigerator.
v. 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.
vi. Representative samples of each batch of plates were examined for sterility by incubating at 30-35°c for 24 hours.

Preparation of well

The wells were made using cork borer on N-agar plate. The borer was deeped 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 35^0 C until it achieves turbidity 1-2 x 10^8 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 over night broth culture or other unstandardized inoculums for streaking plates.

Inoculums of plant extract into the well

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

i. Different concentration of plant extract in (10µl, 20µl.……up to 100µl) to the tube to respective tubes were added.
ii. 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.
iii. 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.

Phytochemical Tests

I. Test for steroid

Salkowski Test

Chloroform solution of the extract when shaken with concentrated sulphuric acid and on standing yields red colour.

II. Test for Triterpene

Salkowski test

Chloroform solution of the extract when shaken with concentrated sulphuric acid, lower layer turns to yellow on standing.

III. Test of Alkaloids

The extracts were mixed with ammonia and then extracted with chloroform solution. To this dilute hydrochloride acid was added. The acid layer was used for chemical tests for alkaloids.

Hager's Test

(Saturated solution of picric acid): The acid layer with Hager's reagent gives yellow precipitate.

IV. Test for Tannins

Gelatin test

Extracts mixed with few drops of 1% solution of gelatin containing 10% sodium chloride gives white precipitate.

V. Test for Flavonoids

Lead acetate tests

Alcoholic solution of the extracts mixed with few drops of 10 % lead acetate gives yellow precipitate.

VI. Test for Lactones

Baljel's tests

The extracts mixed with solution of sodium picrate give yellow orange colour.

VII. Test for Diterpenes

Copper acetate test

The extracts, mixed with solution of copper acetate gives green colour.

VIII. Test for Glycosides

Kellar Killani's test

Dissolve the extract in water with Glacial acetic acid and ferric chloride and concentrated sulphuric acid. They give brown ring at the junction.

IX. Test for Saponins

Foam test

A small amount of extract is shaken with little quantity of water. The foam produced persists for 10 min. It confirms the presence of saponins.

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 concentration. The test fungus was *Candida albicans.*

Table 2

Concentration of each extract used to check antifungal activity

Sr. No.	Plant Sample	Water (Conc. mg/ml)	Acetone (Conc. mg/ml)
1	Sandalwood	7.5	8.3
2	Arjuna bark	19.8	19.9

Results of diameter of inhibition zone (DIZ value)

The measured DIZ of various extracts of plants with different solvents against *C. albicans* are shown in table 3.

Table 3

Results of diameter of inhibition zone

Sr. no	Extract	Diameter of inhibition zone (DIZ) (mm)	
		Acetone extract	Water extract
1	Sandalwood	17.9	12.9
2	Arjuna bark	23.3	_

Results of determination of MIC value

After evaluating the DIZ values extracts (Sandalwood and Arjun bark extract prepared with acetone) were taken which shows higher antifungal activity for MIC test by taking a different concentration (Table 4). The test fungus was inoculated in different concentration of plant extracts i.e.10µl, 20µl, …….100µl.

Table 4
Results of MIC for acetone extracts

Sr. No.	Extracts	Different volume of plant extracts (µl)									
		10	20	30	40	50	60	70	80	90	100
1.	Sandalwood	+	+	+	-	-	-	-	-	-	-
2.	Arjuna bark	+	+	+	+	+	-	-	-	-	-

The growth of *C. albicans in* Sandalwood extract in acetone was seen below 40µl concentration. So, 40µl can be said MIC of Sandalwood extract. The growth of *C. albicans* in Arjun bark extract in acetone was seen below 60µl concentration. So, 60µl can be said MIC of Arjun barks extract.

Phytochemical Test

The results of qualitative screening of phytochemical components in revealed the presence of alkaloids, steroid, glycosides, triterepenes, diterpines, saponin and flavonoids were present in both plants while tannin is extra chemical present in Arjun plant (Table 5).

Table 5
Result of phytochemical test

Sr. No.	Phyto-chemical	Sandalwood	Arjuna bark
1	Steroid	+	+
2	Triterepenes	+	+
3	Alkaloids	+	+
4	Tannin	_	+
5	Flavonoids	+	+
6	Lactones	_	_
7	Diterpines	+	+
8	Glycosides	+	+
9	Saponin	+	+

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. Sandalwood and Arjuna bark extract both shows antifungal activity. *C. albicans* was strongly inhibited by the dry Arjuna bark extract prepared by acetone as solvent followed by Sandalwood extract prepared by acetone as solvent. This suggests that plant 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 plants and their extracts was due to the presence of several primary and/or secondary metabolites such as phenolics, polyphenols, tannins, quercetin, flavones, flavonols, alkloids, terpenoids, lectins, polypeptides, 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 plants Sandalwood and Arjuna were used for antifungal screening against *Candida albicans*. The plant materials were extracted with acetone. 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, tannin, flavnoids, lactones, diterpines, giycosides, sapponins. The result obtained in this study clearly demonstrate broad spectrum antifungal activity of Sandalwood and Arjuna bark extract against *Candida albicans.* The presence of phyto-compound in the extracts including, (steroid, triterpenes, alkaloids, tannin, flavnoids, lactones, diterpines, giycosides, sapponins) may be responsible for these activities. The acetone extracts of plant are more efficient as compared to the water extract.

REFERENCES

1. Manolakaki D, Velmahos G, Kourkoumpetis T, Chang Y, Alam HB, De Moya MM, & Mylonakis E, *Candida* infection and colonization among trauma patients. Virulence, 1(5): 367-375, (2010).
2. Kourkoumpetis, Themistoklis K, The effect of cumulative length of hospital stay on the antifungal resistance of *Candida* strains isolated from critically ill surgical patients. Mycopathologia, 171(2): 85-91, (2011).
3. Fugelsang K, EdwardsC, Wine Microbiology Second Edition, Springer Science and Business Media , New York, ISBN 0387333495, 3–28, (2010)
4. Gurjeet Singh, Raksha AD, Urhekar, Candidal infection: epidemiology, pathogenesis and

recent advances for diagnosis, material and methods. Bulletin of Pharmaceutical and Medical Sciences, 1(1): 1-8, (2013).
5. Mahesh B and Satish S Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Sciences, 4: 839-843, (2008).
6. Raja Ratna Reddy, Krishna Kumari C, Lokanatha O, Mamatha S, Damodar Reddy C, Antimicrobial activity of *Azadirachta Indica* (neem) leaf, bark and seed extracts, Int. J. Res. Phytochem. Pharmacol., 3(1): 1-4 (2013).
7. Shinde Vidya and Dhale DA, Antifungal properties of extracts of *Ocimum tenuiflorum* and *Datura stramonium* against some vegetable pathogenic fungi. Journal of Phytology, 3(12): 41-44, (2011).
8. Hire KK and Dhale DA, Antimicrobial Efficacy and Insilico ADMET Prediction of *Santalum album* L. International Journal of Pharma and Bio Sciences, 3(4): P-727-734, (2012).
9. Kirtikar KR, Basu BD, Indian Medicinal Plants. Vol. I-IV, Lalid Mohan Basu Publication,New Delhi, India, (1975)
10. Le Grand A, Wondergem PA, Verpoorte R, Pousset JL, Anti-infectious phytotherapies of the tree-savannah of Senegal (West-Africa) II. Antimicrobial activity of 33 species. J. of Ethnopharmacol., 22(1): 25–31, (1988).
11. Onkar D, Dhingra and James B, Basic plant pathology method, CRC press, Inc, USA, pp 287-305 (1995)
12. Baron EJ, Finegold SM, Peterson LR, Bailey, Scott's diagnostic microbiology, 9th ed. Mosby, USA, (1994)
13. Koneman EW, Allen SD, Janda WW, Sehreken PC, Winn WC, Diagnostic Microbiology, 4th ed. J. Lippincott Company, Philadelphia, (1992)
14. Mahmoudabadi AZ, Dabbagh MA, Fouladi Z *In vitro* anti-*Candida* activity of *Zataria multiflora* Boiss. J. Evid Based Complement Alternat. Med., 4(3): 351-353, (2007).