ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Selected Medicinal Plants for Anticandida Potential

Kavita Salkar*, Ashish Suthar, Vijaysingh Chauhan, and Vinayak Naik

Piramal Healthcare Limited, Nirlon Complex-1, Off Western Express Highway, Goregaon (east), Mumbai-400063, Maharashtra, India

ABSTRACT

The emergence of resistant strains of microorganisms to existing antimicrobial agents has attracted the focus of Scientists world over towards the alternative sources like natural agents of plant origin. Candida genus is a commensal microorganism, however can turn pathogenic in certain predisposing conditions. Nowadays the resistant strains of Candida are reported in increasing numbers. The purpose of this study was to evaluate the potential of certain plant species against Candida species. Twenty plants belonging to various botanical families and extracted in different solvents were studied against three Candida species by agar-well diffusion method. Seven plants were found to be active against the normal and resistant strains of Candida.

Keywords: anti candida; antimicrobial resistance; plant extracts; agar-well diffusion

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Candida albicans is both a ubiquitous commensal and an important opportunistic human pathogen causing common ailments such as thrush and vaginitis, as well as chronic conditions in immunocompromised patients [1]. The opportunistic fungus *C. albicans* is a major cause of oral and esophageal infections in immunocompromised patients such as human immunodeficiency virus (HIV)-infected individuals, cancer patients and the elderly. Other conditions predisposing individuals to oral *C. albicans* infection include hyposalivation, diabetes mellitus, prolonged use of antibiotics or immunosuppressive drugs, use of dentures, and poor oral hygiene. [2]. *C. albicans* is still considered the major etiologic agent in this infection and accounts for 70% to 80% of organisms isolated from oral mucosal lesions. However, in recent years, *C. glabrata* has emerged as a notable pathogenic agent in the oral mucosa, either as a co-infecting agent with *C. albicans* or as the sole detectable species from oral lesions .In addition, *C. glabrata*-associated oropharyngeal candidiasis infections in HIV and cancer patients tend to be more severe and more difficult to treat than infections due solely to *C. albicans.* [3]

The clinical significance of the oral candidiasis, which is not life-threatening but causes significant morbidity in patients, is increasing with time. Some drugs such as azole antifungal agents are used for chemotherapy of this fungal infection. HIV-positive patients receiving the new therapy (highly active antiretroviral treatment) demonstrate significantly fewer episodes of oral candidiasis than those without highly active antiretroviral treatment However, long-term treatment with antifungal drugs still causes the appearance of drug-resistant *Candida* or side effects [2]. This rise in the appearance of *Candida albicans* and non-albicans species turning resistant to existing antifungal agents has caused the scientist to screen alternative sources of compounds from natural products.

Plants produce a great deal of secondary metabolites, many of them with antifungal activity. Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates [4]. Interest in the therapeutic use of non-conventional, nonprescription, or so-called natural medicinals in the field of infectious diseases has increased remarkably in recent years, mostly driven by the well-known side effects of conventional drugs as well as by the spread of antimicrobial resistance to otherwise efficacious and well-tolerated drugs [5]. Consumers are increasingly interested in complementary and alternative medicines, including herbal medicine, as they perceive these forms of healing as being both safe and effective. This trend in use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants. In the US, a number of medicinal plants have been documented as important source of bioactive compounds [6].

The rapid increase in fungal infections and the growing number of resistant strains indicate an increase need of extensive research in the field of role of herbs against resistant strains. In consideration of the vast potential of plants as antimicrobial agents, few plants belonging to different regions of India were investigated against the various Candida species in

our present study. These plants are known for some of their antimicrobial effects in the previous studies done and folk or traditional medicine.

MATERIALS AND METHODS

Plant material

Plant parts were collected from different regions of India. Thorough literature study and Ethanobotanical data was used to select the plants species studied over here. The plants data along with their botanical family and parts used are mentioned in the table no.1.

Extraction of plant material

The plant materials used in the study were in the form of finely ground powder. The plant parts were air dried and pulverized using a mechanical blender. The dried powdered plant materials were then extracted individually using methanol, hydromethanol and water. The resulting extracts were filtered and dried under reduced pressure to obtain solid extracts. The stock solution of 20 mg/ml of each extract was prepared in methanol. Various dilutions were further prepared to get a series of concentration.

Microorganisms

Resistant and sensitive strains of various species of Candida were used in the study. The test organisms included lab strain of *Candida albicans*- fluconazole resistant, *Candida albicans* ATCC 10231, *Candida krusei* $G03^{FR}$-fluconazole resistant, *Candida krusei* $G06^{FS}$- fluconazole sensitive, *Candida glabrata* $H04^{FS}$ -fluconazole sensitive and *Candida glabrata* $H05^{FR}$ - fluconazole resistant. All the strains were clinical isolates obtained from various hospitals of Mumbai, India and maintained in lab. except *Candida albicans* ATCC 10231.

Agar-well diffusion method

Fungal cultures of Candida strains were cultured overnight at 37°C in Sabourauds agar medium. Colonies from subcultures were suspended in saline and vortexed to give an optical density of 1.0 at 530nm (A_{530nm} 1.0 ≈ 10^8 cfu/ml). This yielded approximately 1.5 x 10^8 cells/ml. The predefined volume of inoculum obtained by above method was mixed with molten Sabourauds agar at 40^0C and poured into sterile petri plates. Plates were allowed to set and solidify. Then wells (six mm in diameter) were cut from the agar using a sterile well-borer and 0.05ml of extract solution was delivered into them. After incubation for 24 hours at 37°C, all plates were examined for any zones of growth inhibition, and the diameters of these zones were measured in millimeters. Positive control of Ketoconazole (10 mcg/ml) and solvent control were included in the study.

ISSN: 0975-8585

RESULTS AND DISCUSSION

The emergence of more and more resistant strains of Candida has led the researches to do more study of natural antifungal agents. Our study involves the screening of three different extracts of twenty plants collected from India (Table 1).

Table 1: Plants studied along with their family name

Sr.No.	Plant name	Family
1	*Alpinia officinarum* Hance	Zingiberaceae
2	*Aegle marmelos* (L.) Correa	Rutaceae
3	*Herpestis monnieri* (L.) Rothm.	Scrophulariaceae
4	*Oroxylum indicum* (L.) Kurz.	Bignoniaceae
5	*Rubia cordifolia* L.	Rubiaceae
6	*Exacum bicolor* Roxb.	Gentianaceae
7	*Baliospermum montanum* (Willd.) Müll.Arg.	Euphorbiaceae
8	*Solanum indicum* L	Solanaceae
9	*Smithia germinifloria* L	Leguminosae
10	*Tribulus terristris* L	Zygophyllaceae
11	*Canscora diffusa* (Vahl) R.Br. ex Roem. & Schult.	Gentianaceae
12	*Coccinia indica* Wight & Arn.	Cucurbitaceae
13	*Peristrophe bicalyculata* (Retz.) Nees	Acanthaceae
14	*Pluchea lanceolata* (DC.) C.B.Clarke	Asteraceae
15	*Holostemma rhedianum* Spreng	Apocynaceae
16	*Picrorhiza kuska* Royle	Scrophulariaceae
17	*Symplocus racemosa* Roxb.	Symplocaceae
18	*Clerodandrum serratum* (L.) Moon	Verbenaceae
19	*Tephrosia purpurea* (L.) Pers.	Fabaceae
20	*Pergularia pallida* L.	Asclepiadaceae

The results clearly demonstrate that the studied plants exhibited significant anti Candida activity. Total twenty plants were studied against Candida species and seven were found to be active at different concentrations. The three different solvent extracts studied once again demonstrated that the methanol extract has broader spectrum of activity followed by aqueous-methanol. The results of anti candida activity of seven active plants are presented in table no.2. *Alpinia officinalis* was the most active plant obtained. All the three extracts studied for this plant were active against all the species of Candida. Thus this plant showed broad spectrum activity and was active not only against *Candida albicans* but also against other species of Candida like *C.krusei* and *C.glabrata* which now a days are causing various serious infections and are being developing resistant to common antibiotics. *A. officinalis* has been studied extensively for its anti microbial and other pharmacological properties. Study carried out by Srividya et al on this plant has shown activity against *C.albicans* strains [7]. In our study the plant was active against the various Candida species including the resistant strains. Another paper of Indrayan et al demonstrates anticandida activity of Alpinia oil [8]. The plant belongs to Zingiberaceae family and its rhizome oil has anti inflammatory activity too. This data strengthen

by our results, which show activity against the resistant strains of Candida, signifies *A.officinalis'* potential as an anticandida agent.

Table 2: Anti candida activity of seven active plants

Plant name	Extract	Conc. mg/ml	CA (l)	CA (a)	CK (GO3)	CK (GO6)	CG (HO4)	CG (HO5)
Alpinia officinarum	MeOH	10	13	12	13	12	11	11d
	MeOH	20	14	12	13	13	11	11d
	HM	10	12	12	12	12	11	11d
	HM	20	13	12	13	13	11	11d
	AQ	10	13	11	11d	-	-	-
	AQ	20	12	11	12	10	10	10
Herpestis monnieri	MeOH	10	12	-	11	9	-	12
	MeOH	20	13	-	12	12	11	14
	HM	10	17	12	16	15	13	18
	HM	20	19	14	18	17	17	19
	AQ	10	-	11d	-	-	-	-
	AQ	20	-	11d	-	-	-	-
Aegle marmelos	MeOH	10	9	10	8	-	-	-
	MeOH	20	11	10	8	-	-	-
	HM	10	14d	10d	-	8	-	-
	HM	20	17d	10d	-	10d	-	-
Oroxylum indicum	MeOH	10	15	11	12	-	10	-
	MeOH	20	16	12	13	-	11	-
Rubia cordifolia	MeOH	10	12	11	13	12	11	12d
	MeOH	20	14	12	14	13	11	12d
Exacum bicolor	AQ	10	12	11d	-	-	-	-
	AQ	20	14	11d	-	-	-	-
Baliospermum montanum	MeOH	10	11d	11d	11	-	-	-
	MeOH	20	13d	11d	12	-	-	-
	HM	10	-	11d	12d	-	-	-
	HM	20	-	11d	12d	-	-	-

Key: MeOH-Methanol extract, HM-Hydro-Methanol extract, AQ-Aqueous extract, CA (l)- *Candida albicans*, CA (a)- *Candida albicans* ATCC10231, CK (GO3)- *Candida krusei* GO3, CK (GO6)- *Candida krusei* GO6, CG (HO4)- *Candida glabrata* HO4, CG (HO5)- *Candida glabrata* HO5, d-diffused zone of inhibition, numbers indicate diameter of zone of inhibition in mm

Herpestris monneira was the next plant amongst the studied plants to demonstrate activity against all the species of Candida. Unlike Alpinia the aqueous extract of this plant was active against *C.albicans* only. The other two solvent extracts viz. methanol and hydromethanol were active against all the species tested. The hydromethanol extract was more active than the methanol extract in terms of diameter of zone of inhibition. The activity was same against the normal as well as the resistant strains of Candida. *H. monneira* has been mentioned in the Ayurveda as a useful plant for various ailments and many reports on its numerous activities including antimicrobial potential has been reported previously [9]. Ethanolic extract of the aerial parts of this plant has shown activity against *C.albicans* in the previously reported studies

[10]. Our study emphasis on its anticandida activity solely where the plant has shown real potential by displaying activity against various resistant Candida species and thus justifies its use in ethanomedicine.

The methanol and hydromethanolic extracts of *Aegle marmelos* were found to be active against *C.albicans* strains. Slight activity was observed for *C.krusei* whereas no activity was recorded for *C.glabrata* strains. *A.marmelos* is known to contain terpenoids which act as an antifungal agent and also it is being used in traditional medicine for its medicinal value [11]. The antibacterial and anti dermatophyte activities of this plant are already established [12]. Its antifungal property was proved in our study where it showed activity against the tested Candida species.

The methanol extracts of *Oroxylum indicum* and *Rubia cordifolia* showed broad spectrum anticandida activity. The other two extracts of these plants were not active against the tested species. *O.indicum* is known for its medicinal properties. It is extensively used in Ayurvedic and tribal medicines. A study carried out by Das S. et al proves its antibacterial properties [13]. The anticandida activity of this plant in our study validates its therapeutic potential mentioned in ancient literature. *R.cordifolia* is known for its activity against phytopathogens also in one of the study carried out by Basu S. et al the plant has shown significant antibacterial activity [14]. Our results add one more property of also being anticandidal in nature in the antimicrobial spectrum of *R.cordifolia.*

The plant *Exacum bicolor* showed activity against the Candida species by its aqueous extract. The other two extracts of this plant did not show any activity. The results are in contrast to the results obtained by other co-workers where the methanol extract of this plant has shown activity [15]. The plant is used in traditional medicine for skin and eye infection and hence was selected here for anticandida activity.

Baliospermum montanum was studied for its antimicrobial properties by other authors but no anticandida activity was recorded [16]. Our plant extract showed slight activity against *C.albicans* and *C.krusei* in methanolic and hydro-methanolic extracts.

Our results of the active plants, probably explains the use of these plants by the indigenous people against a number of infections. The study demonstrates that the active plants have potential as anticandida agents and they can be of importance in medicine as alternative agents.

REFERENCES

[1] Cowen LE, Sanglard D, Calabrese D, Sirjusingh C, Anderson J and Kohn L. J Bacteriol 2000; 182(6):1515-1522

[2] Takakura N, Wakabayashi H, Ishibashi H, Teraguchi S, Tamura Y, Yamaguchi H. et al. Antimicrob Agents Chemother 2003; 47 (8): 2619-2623

[3] Li L, Reding S and Dongari-Bagtzoglou A. J Dent Res 2007; 86(3): 204-215

[4] Quiroga EN, Sampietro AR and Vattuone MA. J Ethanopharmacol. 2001;74: 89- 96

[5] Mondello F, Bernardis F, Girolamo A, Salvatore G, Cassone A. J Antimicrob Chemother 2003; 51: 1223-1229
[6] Wendakoon C, Calderon P and Gagnon D. Journal of Medicinally Active Plants 2012; 1(2): 60- 68
[7] Srividya AR, Dhanabal SP, Misra VK and Suja G. Indian J Pharm Sci 2010; 72(1): 145-148
[8] Indrayan AK, Garg SN, Rathi AK and Sharma V. Indian J Chem 2007; 46B: 2060-2063
[9] Sampathkumar P, Dheeba B, Vidhyasagar V, Arulprakash T and Vinothkannan R. Int J Pharmacol 2008; 4(3): 230-232
[10] Ghosh T, Maity TK, Bose A, Dash GK and Das M. Indian J Pharm Sci 2007; 69 (2): 312-314
[11] Balakumar S, Rajan S, Thirunalasundari T and Jeeva S. Asian Pac J Trop Biomed 2011: 309- 312
[12] Suresh K, Senthilkumar PK and Karthikeyan B. J Phytol 2009; 1(5): 323-327
[13] Das S and Chaudhary MD. Assam University J Science and Technlogy: Biological and Environmental Sciences 2010; 5(I) : 95-99
[14] Basu S, Ghosh A and Hazra B. Phytother Res 2005; 19: 888–894
[15] Paulsamy S and Jeeshna MV. Res J Pharm Biol Che Sci 2011; 2 (4) : 447-457
[16] Vaghasiya Y and Chanda SV. Turk J Biol 2007; 31: 243-248.