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Prosopis gladulosa Medicinal Plant An Alternative Medication Against Clinical Pathogen *Candida albicans*

Tariq Ahmad Lone[1]*, U. Priya[1], S. Menaka[1], M. Sivasankari [1] and Reyaz Ahmad Lone[2]

[1] Department of Microbiology, Sree Amman Arts and Science College, Erode-638102, Tamil Nadu, India

[2]Department of Biotechnology, Periyar University, Salem-636011, Tamil Nadu, India

*Corresponding Author E-mail: tariqtasin@gmail.com

ABSTRACT

Candida albicans is an opportunistic pathogen morphologically yeasts like fungi are responsible to cause candidiasis in women of reproductive age. Therefore this present study focused on antifungal activity of Prosopis gladulosa against Candida albicans strains isolated from patients. The samples were isolated from the patients of admitted in the hospital at Erode. It was identified as Candida albicans by the morphologically and biochemically tests from the 35 samples six different strains of Candida albicans were isolated. The antifungal susceptibility tests were done against different antifungal agents as the six isolated strains were resistant to Fluconazole, Itraconazole, Nystatin and Clotrimazole, and it was found that all the strains were sensitive to Amphotericin B. The strain 4, 5, 6 are also sensitive Ketoconazole in addition to Amphotericin B therefore it was concluded that all the six strains were multidrug resistant. The plant used in this was Prosopis gladulosa showed antifungal against six different strains of Candida albicans. In that acetone extracts, methanol, petroleum ether extract and water extract antifungal and increased with increase in concentrations. Prosopis gladulosa aqueous extracts does not have much activity against Candida albicans strains.

Key words: *Medicinal plant, Plant extracts, Alternative treatment, Prosopis gladulosa, Anti-yeast activity*

INTRODUCTION

Candida species are thin walled; ovoid yeast cells typically 4-6 cm in size, often form the normal flora of skin, mouth, vagina, intestine and known to be opportunistic pathogens infect persons with low body defense mechanism[1]. Candidiasis is the infection caused by the species of genus *Candida* which can be acute or chronic, superficial or deep and its clinical symptoms are so wide that a more specific definition cannot be made[2,3]. Various identification tests like growth pattern, sugar fermentation, urease production and development of germ tube in human serum confirms the presence of *Candida albicans*[4]. *Candida* is harmless initially, but may become pathogenic when the conditions in the vagina change, particularly when there is a rise in vaginal pH or an excess of glycogen that has not been converted to lactic acid by *Lactobacilli* [5]. Patients usually has a history of HIV infection, diabetes mellitus, drug addiction or inhaled steroids, patient with low body defense mechanisms a broad spectrum antibiotics were risk cases for *Candida* infections[6,7]. Antifungal susceptibility of Vaginal yeast isolates in a rural community of India, since majority of *Candida albicans* isolates were susceptible to Fluconazole, its use may be continued for empirical therapy of uncomplicated candidal vulvovaginitis in the community[8]. Plants have been used as source of medicine since the dawn of civilization established tested drugs from medicinal plants heals

various diseases and disorders to which there is no solution from medicine even today and hence ethanotherpeutics has been reported[9]. *Prosopis gladulosa* is commonly used to treat infections, open wounds, dennatological ailments, anti-acid can treat digestive problems and its aqueous extract are antibacterial as well as antiseptic properties[10]. The phytochemicals of *Prosopis gladulosa* such as S-hydroxytryptamine have anti-depressant activity, apigenin have anti-allergic, anti-bacterial, anti-dermatic, anti-inflammatory and anti-viral activity, isorhamnetin-3-digluciside has hepatoprotective activity, L-arabinose and quercetin have analgesic, anti-allergic, anti-bacterial, anti-diabetic, anti-inflammatory and anti-viral activity[11]. In this study *Candida albicans* was isolated, identified by morphologically and biochemically and checked out susceptibility test. The plant *Prosopis gladulosa* was selected according to its availability and ethnobotanical significance in the treatment of opportunistic mycosis a choice of treatment.

MATERIALS AND METHODS

Study area

Thirty five vaginal samples were collected from the Hospital in and around at Erode District, Tamil Nadu, India. The married and sexually active women between 18-50 year of age were selected from health care center with self-reported symptoms of vaginal discharge, genital itching and genital burning.

Sampling procedure

Specimens were collected from the lateral wall of vagina of each woman by using of sterile cotton tipped swabs. One of the swab was inoculated into sabaroud's dextrose agar (Himedia Mumbai, india) supplemented with 0.06 g/ml of Gentamycin with 0.5% cyclohexamide[12]. The plates were then incubated at for 30^{o}C for 5 days. The second swab was used to determine the presence of yeast by direct wet mount microscopy by adding a drop of 10% potassium hydroxide solution, which was used for the observation of and yeast cells and hyphae in a light microscope using low magnification power.

Identification of Yeast isolates

Gram's staining

Smooth creamy white colour colonies of strain 1, 2, 3, 4, 5, 6 with yeast odour were selected from sabaroud's dextrose agar medium and subjected to Gram staining then observed under low and high power objectives.

Test for germ tube formation

Germ tube formation test is the confirmatory test for the identification of *Candida albicans* was carried by taking the isolated strains 1, 2, 3, 4, 5, 6 and separately inoculated in to 0.5ml of human serum in small tubes and incubated at 37^{o} C for 3 hours. After incubation a loop full of culture of strains 1, 2, 3, 4, 5, 6 were placed on grease free slides, over laid with cover slip then observed under low and high power objectives to visualize germ tube[13].

Carbohydrate fermentation tests

The isolated strains 1, 2, 3, 4, 5, 6 were further confirmed by subjecting to the carbohydrate fermentation test viz sugars of dextrose, galactose, lactose, maltose, sucrose and trehalose. The 5 ml of carbohydrate solution having pH 7.4 containing 1 % peptone, 1 % sugar, 0.3 % beef extract and 0.5 % NaCl, 0.2 % Bromothymol blue in distilled water medium was dispensed in sterilized Durham tube and 0.2 ml of saline suspension of the test organism was added and incubated at 37^{o}C for 10 days.

Antifungal susceptibility test

Antifungal susceptibility testing was performed by Clinical and Laboratory Standards Institute[14]. An Inoculum was prepared by picking up isolated strains 1, 2, 3, 4, 5, 6 from the Saboraud's dextrose agar medium were suspended in 5ml of sterile saline and its turbidity was adjusted visually with the transmittance to that produced by a 0.5 Mc Ferland standard. After obtaining the 0.5 Mc Ferland standards for isolated strains 1, 2, 3, 4, 5, 6 they were swabbed uniformly on the surface of Muller Hinton agar and allowed for dry under sterile conditions. After drying antifungal discs of Amphotericin B, Clorimazole, Fluconazole, Itraconazole, Ketaconazole, and Nystatin were placed onto the surface of inoculated plates of isolated strains 1, 2, 3, 4, 5, 6. The plates were incubated at 35^{o}C for 24 hours and after incubation zone of inhibition was measured, results were recorded as susceptible or resistant.

Collection of plant materials

The fresh leaves of *Prosopis gladulosa* were collected from in and around of Erode district, Tamil Nadu, India. All the healthy fresh leaves of *Prosopis gladulosa* were cleaned and washed thoroughly five times with running tap water then followed once with distilled water. The washed leaves of *Prosopis gladulosa* were air dried under shaded place within sterile condition[15].

Solvent extraction

Air dried leaves of *Prosopis gladulosa* were milled to a fine powder. The extract was obtained by using cold extraction method. The fine leaves powder of *Prosopis gladulosa* was filled in conical flasks and extracted with aqueous (water), acetone, methanol and petroleum ether extracts at a rate of 1:5. All the respective flasks were incubated for one week at room temperature and gently shaked for 30 minutes at the end of every day. After six days of incubation all the contents in the flasks were filtered through ordinary filter paper and the filtrates were evaporated to dryness under reduced pressure using rotatory evaporator at 4^{o}C. All the extracts were preserved in tightly closed bottles in a refrigerator until used for the antifungal testing[16].

Preparation of crude extracts solution

The crude extracts was prepared be dissolving 100mg of leaves extracts in 1ml of Dimethyl sulfoxide (DMSO) solution. The different concentration of leaves extracts were prepared 10µl, 20µl and 30µl.

Inoculum preparation

All the clinical isolated strains 1, 2, 3, 4, 5, 6 of *Candida albicans* were prepared in 0.85% saline and were incubated on a shaker at 37^{o}C for 18 hours and then diluted to 1:10 the concentration to yield a culture density of approximately 180 CFU/ml[15].

Antifungal assays of plant extract by Agar-well diffusion method

This method is commonly employed for antibiotics sensitivity test and based on the fact that for the given antibiotics, the size of the zone of inhibition is related to minimum inhibitory concentration (MIC). MIC is referred as the lowest concentration of antibiotic that exhibits the zone of inhibition of the assay plates. Muller- Hinton agar medium was (Hi Media Mumbai) prepared, sterilized, poured on to sterilized petriplates and allowed for solidification under sterile condition[15]. The clinical isolated strains 1, 2, 3, 4, 5, 6 of *Candida albicans* were swabbed evenly on the surface of the dried Muller- Hinton agar and plates were allowed to stand for 15 minutes at room temperature under sterile condition. With me help of a sterile cork borer, wells with 6mm diameter were made in each plate. The different *Prosopis gladulosa* leaves extract concentrations such as 10µl, 20µl, 30µl were taken with micropipette and loaded into a separate well in Muller- Hinton agar containing clinical isolated strains 1, 2, 3, 4, 5, 6 of *Candida albicans*. The plates were incubated for 24 hours at 37^{o}C and the observations were recorded. The zone of inhibition or halo like area was measured and recorded. One well with DMSO was used as negative control[17].

RESULTS

The taxonomic classification of medicinal plant *Prosopis gladulosa* belongs to Kingdom-Plantae, Subkingdom- Tracheoionata, Division-Mangoliophyta, Class-Magnoliopsida, Subclass- Rosidae, Order-Fabales, Family-Fabaceae, Genus- *Prosopis* and Species- *Prosopis gladulosa*

Isolation and Identification

Among the 35 isolates only six showed the positive characteristic similar to *Candida albicans.* In gram staining most of the isolated samples showed large Gram positive ovoid cells. They are thin walled yeast cells. Then the suspected isolated strains 1, 2, 3, 4, 5, 6 were confirmed by Germ tube formation and Carbohydrate fermentation tests (Table 1).

Antibiotic susceptibility test for five strains of *Candida albicans*

Candida albicans strain 1 showed resistant against Clotrimazole, Fluconazole, Itraconazole, Nystatin but showed sensitivity to Amphotericin-B and Ketaconazole while *Candida albicans* Strain 2 showed resistant against Clotrimazole, Fluconazole, Itraconazole, Ketaconazole, Nystatin but sensitivity to Amphotericin-B (Table 2). *Candida albicans* strain 3 showed resistant against Clotrimazole, Fluconazole,

Itraconazole, Ketaconazole, Nystatin but sensitivity to Amphotericin-B while *Candida albicans* strain 4 Clotrimazole, Fluconazole, Itraconazole, Nystatin but showed sensitivity to Amphotericin-B and Ketaconazole (Table 3). *Candida albicans* strain 5 showed resistant against Clotrimazole, Fluconazole, Itraconazole, Nystatin but showed sensitivity to Amphotericin-B and Ketaconazole while *Candida albicans* strain 6 showed resistant against Clotrimazole, Fluconazole, Itraconazole, Nystatin but showed sensitivity to Amphotericin-B and Ketaconazole (Table 4). The results revealed that the all six strains were multidrug resistant.

Anifungal assay of *Prosopis gladulosa*

For the present *Prosopis gladulosa* medicinal plants were used to screen its antifungal activity against *Candida albicans.* The Table 5 showed the results of the antifungal activity of *Prosopis gladulosa* against six different strains of *Candida albicans. Candida albicans* strain 1, 2, 5 and 6 did not showed susceptibility activity in the methanol, acetone, petroleum ether and water extracts.

Table 1. Morphological and Carbohydrate Fermentation tests for the isolated strains from vaginal samples

Tests	Vaginal sample strain of *Candida albicans*					
	S1	**S2**	**S3**	**S4**	**S5**	**56**
Gram's staining	+	+	+	+	+	+
Germ tube test	+	+	+	+	+	+
Dextrose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Lactose	-	-	-	-	-	-
Maltose	+	+	+	+	+	+
Sucrose	-	-	-	-	-	-
Trehalose	+	+	+	+	+	+

Note where: + stands for positive reaction and – denotes negative reaction

Table 2. Antibiotic susceptibility test for isolated stains *Candida albicans* strain1 and strain 2

Antibiotics used	Strength Antibiotics Discs (mg)	Zone of inhibition (mm) in strain 1	Results of S1	Zone of inhibition (mm) in strain 2	Results of S2
Amphotericin B	100	17	S	18	s
Clotrimazole	10	08	R	02	R
Fluconazole	10	23	R	03	R
Itraconazole	10	09	R	01	R
Ketoconazole	10	32	S	01	R
Nystatin	100	15	R	09	S

Note were: S-denotes sensitive and R-resistant of strains against respective antibiotics

Table 3. Antibiotic susceptibility test for isolated stains *Candida albicans* strain 3 and strain 4

Antibiotics used	Strength Antibiotics Discs (mg)	Zone of inhibition (mm) in strain 3	Results of S3	Zone of inhibition (mm) in strain 4	Results of S4
Amphotericin B	100	18	S	17	s
Clotrimazole	10	01	R	-	R
Fluconazole	10	01	R	01	R
Itraconazole	10	-	R	-	R
Ketoconazole	10	-	S	01	R
Nystatin	100	15	R	09	S

Note were: S-denotes sensitive and R-resistant of strains against respective antibiotics

Table 4. Antibiotic susceptibility test for isolated stains *Candida albicans* strain 5 and strain6

Antibiotics used	Strength Antibiotics Discs (mg)	Zone of inhibition (mm) in strain 5	Results of S5	Zone of inhibition (mm) in strain 6	Results of S6
Amphotericin B	100	15	S	18	S
Clotrimazole	10	-	R	-	R
Fluconazole	10	-	R	-	R
Itraconazole	10	01	R	01	R
Ketoconazole	10	-	S	-	R
Nystatin	100	17	R	17	S

Note were: S-denotes sensitive and R-resistant of strains against respective antibiotics

Table 5. Antifungal susceptibility assay of *Prosopis gladulosa* against vaginal isolated stains1, strain 2, strain 3, strain 4, strain 5, strain 6 of *Candida albicans*

Strain	Concentration (mg)	Solvent used and diameter of zone of inhibition (mm)			
		Methanol	Acetone	Petroleum ether	Water
Strain 1	10 mg	-	13mm	-	-
	20 mg	-	17mm	-	17mm
	30 mg	-	24mm	-	21mm
	40 mg	-	26mm	-	25mm
	50 mg	15 mm	27mm	-	-
Strain 2	10 mg	-	-	-	-
	20 mg	-	-	-	-
	30 mg	-	-	-	-
	40 mg	-	-	-	-
	50 mg	-	19mm	16mm	13 mm
Strain 3	10 mg	16mm	-	-	-
	20 mg	18mm	-	-	-
	30 mg	24mm	-	-	-
	40 mg	27 mm	-	9mm	-
	50 mg	29mm	10mm	10 mm	10mm
Strain 4	10 mg	-	13mm	17mm	-
	20 mg	-	15mm	21mm	-
	30 mg	-	21mm	25mm	-
	40 mg	9 mm	21mm	27mm	9 mm
	50 mg	11 mm	-	30mm	11 mm
Strain 5	10 mg	-	-	18mm	-
	20 mg	-	-	22mm	-
	30 mg	-	-	24mm	-
	40 mg	-		12 mm	-
	50 mg	-		26mm	-
Strain 6	10 mg	-	-	-	-
	20 mg	-	-	-	-
	30 mg	-	-	-	-
	40 mg	-	13mm	13mm	-
	50 mg	-	14 mm	28mm	15mm

DISCUSSION

The suspected strains of *Candida albicans* isolates were grown on the corn meal agar and showed the formation of large, refractive and thick walled terminal chlamydospore[18]. *Candida* species may be either a commensal or pathogen of the vagina, infact which indicates that changes in the vaginal micro environment a generally necessary for *Candida* to induce pathological changes associated with clinical

symptoms. Over expression of the multidrug transporter, *Candida* drug resistanceis due to protein1 a member of the ATP Binding Cassette transporter super family and account for a clinically significant mechanisms of azoles resistance in the pathogenic yeast *Candida albicans*[19]. Prevalence of *Candida albicans* infections is approximately three-quarters of all women experience at least one episode of vulvovaginal candidiasis during their lifetime nearly half of them suffer from multiple episode[20]. Medicinal plants as a sources relief from illness can be treated back over few milleni to written documents of the early civilization in China, India and Near east, but it is doubtless and art as old as mankind[21]. Neanderthals living 60,000 years ago used medicinal plants, in present day Iraq used plants such as holly back these plants are widely used in ethanomedicine around the world. *Prosopis gladulosa* is commonly used to treat infections, open wounds and deontological ailments have been extensively in herbal medicine in many tropical and subtropical countries[22]. An increasing prevalence of infections caused by newer emerging fungal pathogens has been detected in humans and the population of patients at risk has expanded to include those with a broad list of medical conditions[23]. Many of the drugs currently available have undesirable effects or very toxic. Azoles are fungi static and not fungicidal, so it has given rise to both primary and secondary drug resistance. Therefore there is a real need for a next generation of safer and more potent antifungal agents. One possible approach is to screen local medicinal plants to get the compound which can be directly used as antifungal agents or can serve as template for drug development[24]. Vulvovaginal candidiasis is an important cause of morbidity in women of reproductive age and majority of the infection is caused by the species *Candida albicans*. In about five percentages of cases, the disease has a chronic course showing frequent and refractory episodes[25]. Therefore this present study focused on antifungal activity of *Prosopis gladulosa* against *Candida albicans* strains isolated from patients. The samples were isolated from the patients of admitted in the hospital at Erode. It was identified as *Candida albicans* by the morphologically and biochemically tests from the 35 samples six different strains of *Candida albicans* were isolated[26]. The antifungal susceptibility tests were done against different antifungal agents as the six isolated strains were resistant to Fluconazole, Itraconazole, Nystatin and Clotrimazole, and it was found that all the strains were sensitive to Amphotericin B. The strain 4, 5, 6 are also sensitive Ketoconazole in addition to Amphotericin B therefore it was concluded that all the six strains were multidrug resistant by comparing with previous literature[27]. Azoles are antifungal drugs frequently used for the treatment of vulvovaginal candidiasis but there is evidence, however of an increased azoles resistance among the isolates of *Candida* species isolated from patients with vulvovaginal candidiasis[28, 29]. In the present study *Prosopis gladulosa* showed antifungal activity of against six different strains of *Candida albicans*, in that acetone extracts, methanol, petroleum ether extract and water extract antifungal activity increased with increase in concentrations by compared with previous literature[30]. *Prosopis gladulosa* has antibacterial activity against several positive bacteria also having analgesic and anti-inflammatory effects[31]. The maximum antifungal activity of *Prosopis gladulosa* plant salt was shown against *Candida albicans* and *Cryptococcus neoformans*, followed by *Aspergillus flavus* in higher concentrations in previous studies in our study we found the similar that *Prosopis gladulosa* showed antifungal activity against all the strains of *Candida albicans* and was more pronounced only in acetone extracts[32]. The antifungal activity when compared to standard antibiotics such as Amphotericin B, Ketoconazole, Itraconazole, Nystatin, Clotrimazole and Fluconazole. Findings from the current study support the use of *Prosopis gladulosa* in traditional medicine for the treatment of various bacterial and fungal infections[33, 34].

CONCLUSION

In this study it was finally concluded that *Prosopis gladulosa* had a good antifungal activity. Further work is required to isolate the bioactive constituents and the antifungal properties of these compounds this may help to find the compound responsible for antifungal activity. It's obvious than more scientific studies need to be conducted as only a fraction of the plants used in traditional medicine have been rigorously tested though modern research. Such studies are also urgently needed because due to the increase in human population and spread of habitat. It is also recommended that once the bioactive compounds have been isolated it should be tested for bacteria, fungi and viruses at a stretch.

REFERENCES

1. Abdur Rahman, A., Samoylenko, V., Melissa, R.J., Rajnish, S., Surendra, K.J., Shabana, I.K., Babu, L.T. and Ilias, M. Antiparasitic and Antimicrobial Indolizidines from the Leaves of *Prosopis glandulosa* var. *glandulosa*, *Planta Med*., **77(14)**: 1639–1643 (2011)
2. Al-Fattani, M.A. and Douglas, L.J. Biofilm matrix of *Candida albicans* and *Candida tropicalis*: chemical composition and role in drug resistance. *J Med Microbiol.*, **55**: 999–1008 (2006)
3. Ahmad, A., Khursheed, A.K., Sabiha, Q. and Viqaruddin, A. Antifungial activity of some hydrosoluble *Prosopis juliflora* alkaloids, *Fitoterapia*., **60**: 86–89 (1989)
4. Angiolella, L., Facchin, M., Stringaro, A., Maras, B., Simonetti, N. and Cassone. A. Identification of glucan associated enolase as a main cell wall protein *Candida albicans* and indirect target of lipopeptide antimycotics, *J of infectious diseases.*,**173**: 684-690 (1996)
5. Bader, T., Bodendorfer, B., Schroppel, K. and Morschhauser, J. Calcineurin is essential for virulence in *Candida albicans. Infect Immun.*, **71**: 5344–5354 (2003)
6. Burkhart, A. A monograph of the genus *Prosopis. J Arnold Arboretum.*, **57**: 450–525 (1976)
7. Calderone, R.A. and Fonzi W.A. Virulence factors of Candida albicans. *Trends Microbiol*., **9**: 327–334 (2001)
8. Changdeo, S.A. Species distribution, virulence factors and antifungal susceptibility profile of *Candida* isolated from Oropharyngeal lesions of HIV infected patients. *Int J Curr Microbiol App Sci.*, **3(1)**: 453-460 (2014)
9. Clinical and Laboratory Standards Institute. Methods for antifungal disc diffusion susceptibility testing of yeast. Approved guidelines M-44A.Wayne, PA:CLSI; 2004.
10. Crowe, J.D., Sievwright, I.K, Auld, G.C., Moore, N.R., Gow, N.A. and Booth, N.A. *Candida albicans* binds human plasminogen: identification of eight plasminogen-binding proteins. *Mol Microbiol* **47**: 1637–1651.
11. Francois, L.M., Duncan, W. and Bernhard, H. *Candida albicans* pathogenicity mechanisms. *Virulence*., **4**(2) 119–128 (2013)
12. Gow, N.A. 1997. Germ tube growth of Candida albicans. *Cuurent Top Med Mycol.*, **8(1-2)**: 43-55.
13. Johnson, M.E., Warnock, D.W, Luker, J., Porter, R.S. and Scully, C. Emergence of azole drug resistance in *Candida* species from HIV-infected patients receiving prolonged fluconazole therapy for oral candidosis. *J Antimicrob Chemother*, **35 (1)**: 103-114 (1995)
14. Kanthasamy, A., Subramanian, S. and Govindasamy, S. Bactericidal and fungicidal effects of Prosopis juliflora alkaloidal fraction. *Indian Drugs.* **26**: 390–394 (1989)
15. Karkowska-Kuleta, J., Rapala-Kozik, M. and Andrzej, K. Fungi pathogenic to humans: molecular bases of virulence of *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*. *Acta Biochimica Polonica*., **56 (2)**: 211–224 (2009)
16. Kothavade, R., Kura, M., Valand, A. and Panthaki, M. Candida tropicalis: its prevalence, pathogenicity and increasing resistance to fluconazole. *J Med Microbiol*., **59**: 873-880 (2010)
17. Lata R Patel, Jayshri D Pethani, Palak Bhatia, Sanjay D Rathod, Parul D Shah. Prevalence of Candida infection and its Antifungal susceptibility pattern in tertiary Care hospital, Ahmadabad. *Nat J Med Res*., **2(4)**: 439-441 (2010)
18. Masia Canuto, M., Guiterrez Rodero, F., Ortiz de la, Tabla, D.V., Hernazdez, A.I., Martin, G.C., Sevillano, A,A. and Martin H. Determinants for the development of oropharyngeal colonization and infection byfluconazole- resistant *Candida* strains in HIV-infected patients. *Eur J Clin Microbiol Infect Dis.*, **19 (8)**: 593-601.
19. Mavor, A.L., Thewes, S. and Hube, B. Systemic fungal infections caused by *Candida* species: epidemiology, infection process and virulence attributes. *Curr Drug Targets.*, **6**: 863–874 (2005)
20. Mohammad Yaseen, K., Saleh, A., Vimal, K. and Shalini, R. Recent advances in medicinal plant biotechnology. *Indian Journal of Biotechnology*., **8**: 9-22 (2009)
21. NCCLS. Approved standard. 2. Vol. 22. National Committee for Clinical Laboratory Standards; 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts; 2. **22**: p. 1-51.

22. Melek. I., Mustafa Altay, A., Ayse Nedret, K., Erkan Y., Omer E., Suleyman, D., Gonca, D. Investigating virulence factors of clinical *Candida* isolates in relation to atmospheric conditions and genotype. *Turk J Med Sci.,* **42 (Sup.2)**: 1476-1483 (2012)
23. Mendes, A., Mores, A.U., Carvalho, A.P., Rosa, R.T., Samaranayake, L.P. and Rosa, E.A. *Candida albicans* biofilms produce more secreted aspartyl protease than the planktonic cells. *Biol Pharm Bull.,* **30**: 1813–1815 (2007)
24. Sardi, J.C., Duque, C., Hofling, J.F. and Goncalves, R.B. Genetic and phenotypic evaluation of *Candida albicans* strains isolated from subgingival biofilm of diabetic patients with chronic periodontitis. *Med Mycol.,* **50**: 467–75 (2011)
25. Samoylenko, V.P., Ashfaq, M.K., Jacob, M.R., Tekwani, B.L., Khan, S.I., Manly, S.P., Joshi, V.C., Walker, L.A. and Ilias, M. Indolizidine, antiinfective and antiparasitic compounds from Prosopis glandulosa var. glandulosa. *J Nat Prod.*, **72**: 92–98 (2009)
26. Segal. E. and Elad. D. *Candida* species and blasto schizomycescapitatus. In: collier. L., Balows. A., Sussman, M., editors Topley and Wilson's microbiology and microbial infections, *Oxford University Press.*, **4(9)**: 423-460 (1998)
27. Shahid. M., Amalik, M., Rizvi, W. and Singhai, M. Protein profile of a fluconazole resistant *Candida albicans* isolated from HIV-1 infected patients: Evaluation of protein extraction methods and development of a simple procedure, *Global Journal of Biotechnology and Biochemistry.*,**1**: 1-6 (2006)
28. Sobel. J.D., Chaim, W., Nagappan, V. and Leamam, D. Treatment of vaginitis caused by *Candida glabarata,* use of tropical boric acid flucytosine. *Am J Obstet Glynecol.,* **189**: 1297-1300.
29. Tariq, A.L. and Reyaz, A.L. Antibacterial activity of *camellia sinensis* leaves against *Staphylococcus aureus* and *Escherichia coli*. *Inter J of Advance Pharm and Biolo Scie*., **3(1)**: 1-7 (2012)
30. Uddin, S.J., Shilpi, J.A., Middleton, M., Shoeb, M., Nahar, M. and Sanker, S.D. Swamalin and Cis-Swamalin, Two new tetrahydrofuran derivates with free radical scavenging activity from the aerial parts of *Cuscuta reflexa, Nat Prod Res.*,**21(7)**: 663-668 (2007)
31. Uniyal, S.K, Singh, K.N., Jamwal, P. and Lal, B. Tradition use of medicinal plants among the tribal communities of Chh.ota bhangal, Western Himalayan, *J Ethnomed,* **2**: 1-14 (2006)
32. Willis, A.M., Coulter, W.A., Fulton, C.R., Hayes, J.R., Bell, P.M. and Lamey, P.J. The influence of antifungal drugs on virulence properties of Candida albicans in patients with diabetes mellitus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.,* **91**: 317-21 (2001)
33. Wu, T., Samaranayake, L., Cao, B. and Wang, J. In-vitro proteinase production by oral Candida albicans isolates from individuals with and without HIV infection and its attenuation by antimycotic agents. *J Med Microbiol*. **44**: 311-316 (1996)
34. Yun-Liang, Y. Virulence factors of Candida species. *Journal of Microbiol Immunol Infect.*, **36**: 223-228 (2003)