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Nosocomial fungal infections and its control measures using medicinal plant of marine origin

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Abstract
The aim of the study was to evaluate the antifungal activity of the extracts of medicinal marine algal plant *Padina gymnosphora* against Candida albicans, a nosocomial fungal strain. The plant was selected on the basis of the previous study report on ethnobotanical uses. Aqueous and methanol extracts of the plant was screened *in vitro* for their antifungal activity against the nosocomial fungus Candida albicans. 100µl concentrations of both extracts were used in well diffusion assay. The study showed the anti-candidial effect, both in methanol and aqueous extracts. The growth inhibition rate of Candida albicans was 12mm in aqueous extract. But the inhibitory zone of methanol extract was seen above 22mm. The standard antibiotics show the inhibition zone as 26mm.The methanol extracts of plant was more efficient as compared to the aqueous extract.

Keywords: padina gymnosphora, candida albicans, well diffusion assay, aqueous extract, nosocomial infection, methanol extract, medicinal plant, marine algae

1. Introduction
Advances in medical and surgical therapy over the past two decades have changed the type of patients cared for in U.S. hospitals. Also, care in specialized units and the use of invasive monitoring devices, parenteral nutrition, broad-spectrum antimicrobial agents, and assisted ventilation have helped to treat patients suffering from previously devastating or fatal diseases and have provided life to premature neonates previously thought to be nonviable (Anaissie, and Bodey. 1989 [5]. These immune compromised patients are highly susceptible to nosocomial infections caused by organisms such as fungi that were previously considered to be of low virulence or "nonpathogenic" (Bross, *et al*., 1989; Chang, *et al*., 1995) [7, 8]. Fungal infections in these patients are often severe, rapidly progressive, and difficult to diagnose or treat (Edwards, 1991) [9]. A thorough appreciation and understanding of fungal infections, including diagnostic and therapeutic modalities, are needed among clinicians and microbiologists to provide better patient care. Prevention methods aimed at reducing identified risk factors for nosocomial fungal infection are being increasingly advocated (Iwata, 1992 and Peacock *et al*, 1993) [10, 11]. Over the past decade, alternatives to amphotericin B, including the azoles fluconazole and itraconazole have become available to treat nosocomial fungal diseases. Most Candida spp. are susceptible to these antimicrobial agents, although there have been some reports of relative resistance among C.lusitaniae and T. glabrata isolates to amphotericin- B (Pfaller *et al*., 1994) [12]. Although Candida spp. have become common human pathogens, relatively few of the more than 100 species of Candida previously identified have been isolated from humans. C. albicans is by far the most common Candida sp. causing infections in humans, followed by C. tropicalis, C. parapsilosis, C. krusei, C. lusitaniae, and T. (C.) glabrata. The majority of nosocomial fungal infections are reported to be caused by Candida spp (Beck-Sague, and Jarvis, 1993) [6]. At hospitals reporting data to the NNIS system during 1980 to 1990, Candida infections accounted for 78.3% of nosocomial fungal infections, followed by Torulopsis glabrata (7.3%) (Sometimes classified as Candida glabrata), and Aspergillus spp. (1.3%) (Schaberg, *et al*., 1991) [13].

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines, with acceptable therapeutic index for the development of novel drugs. 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 (Faizi, *et al*., 2008) [14]. There are plenty of reports on different medicinal plant extracts showing anti-fungal, more specifically anti-candidal activity are available through published works (Leite, *et al*., 2004; Okeniyi *et al*., 2012; Kalpana and Prakash, 2015) [20, 22, 4].

It is in view of this, that the present study was carried out to evaluate the control activity of marine macro algae Padina gymnasphora plant extracs against the the nosocomial fungal isolates of *Candida albicans.*

2. Materials and Methods
Collection of plant
The whole plant part of the marine macro algae *Padina gymnosphora* was collected from Thondi coastal region, East coast of Tamilnadu, India. Samples were cleaned immediatly by sea water and then fresh water. After thorough cleaning the algal plant samples were air dried in the shade at room temperature. Then the dried samples were ground well to a fine powder using a Blender. The powder form of the sample was stored in the container at 4 °C for further experimental use.

Collection of Nosocomial Pathogen
The culture of the Nosocomial pathogenic fungus, *Candida albicans* maintained in the Department of Microbiology, K.A.P.V. Government Medical college, Thiruchirappalli,

Tamilnadu, India was collected for this study. Loopful fungal spores were streaked on PDA medium spreaded plates and incubated at 37°C for 2-3 days. After the growth of fungus in plates it was taken to antifungal assay.

Preparation of extracts

Aqueous extract

The P.gymnosphora powder sample (150 gm) was added in a conical flask containing 850 ml sterile distilled water and stirred well at 6000 rpm for an hour, using a high speed mechanical stirrer the resulting solution was first filtered through whatmann-1 filter paper and sterilized at 121°C for 20 min, and used for assay experiment.

Solvent extract

Likewise, 150gm the sample powder was filled in the thimble and extracted successively methanol using a soxhlet apparatus for 48 hr. The extract was underwent to evaporation of methanol and preserved in an airtight vials at 5°C for further experimental use.

Anti -Candidial assay

For determination of anti-candidial effect or inhibitory effect, Well diffusion assay method was used (Kalpana and Prakash, 2015) [23].

Well diffusion method

Fine cultured isolated colonies of the *Candida albicans* 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 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. After 15 minutes, adjusting the turbidity of the inoculums suspension, loopful of suspension inoculates into flask containing Agar. Mix it well and pour it into plate and rotate the plate for even distribution. Representative samples of each batch of plates were examined for sterility by incubating at 30-35°c for 24 hours.

Inoculation of test extract

In a plate, 3 wells were made for the inoculation of plant extract and standard antibiotics (vancomycin).The wells were made using corn borer. The borer was dipped into the alcohol for sterilization and then was used to make wells. Using micropipette, 100µl of P.gynnosphora extract (consider as a anti-fungal drug) was added to respective wells. The plates were first placed at 4°c for 30 min in order to diffusion of extract and standard antibiotics. Then plates were incubated at 37°c for 48 hours at room temperature. The diameter of the inhibition zones were measured in millimeter at the end of the incubation time.

Statistical Analysis

The values of all the above methods are expressed as Mean ± SEM. Total variation in data was calculated one way analysis of variance (ANOVA) followed by Dunnett's test. Values of $P<0.1$ & $P<0.05$ were considered statistically significant.

3. Results and Discussion

The study showed the anti-candidial effect of both in organic solvent (methanol) extract and aqueous extracts of P.gymnosphora. The growth inhibition of Candida albicans seen below 12mm of the aqueous extract. But greater efficacy of inhibition was showed by methanol extracts of the test sample. The inhibitory zone of methanol extract was seen above 22mm. The standard antibiotics vancomycin shows the inhibition zone as 26mm.The different extract reduced the fungal colony growth of the Candida albicans.

Table 1

Nosocomial fungal species	Zone of inhibition in (mm)			
	Negative control (DMSO only)	**Positive control**	**Experimental**	
		Vancomycin	*P.gymnos phora* aqueous extract	*P.gymnos phora methanolic* extract
Candidia albicans	Nil	26mm	12mm	22mm

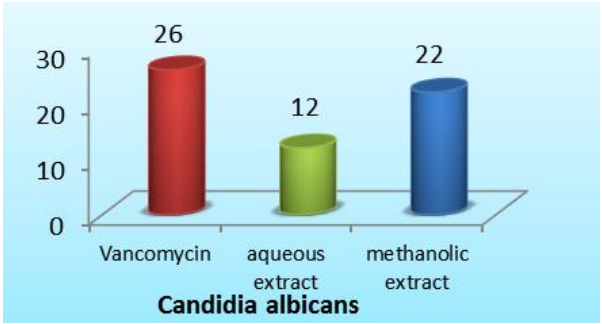

Fig 1

Anti Candidial Activity

The inhibitory effect varied between the extracts of organic solvent and the aqueous extract (Table. 1 Fig. 1). The percentage of the inhibition was almost 89% in the methanol solvent extract at the concentration of 100µl. Aquous extract at 100µl showed 62 % of the inhibition of the C.albicans growth. Our study indicated that the methanol extract of P.gymnosphora showed significantly ($p<0.01$) more potent inhibitory activity against Candida albicans than aquous extract.

Many studies indicated that the plant extracts such as Acacia nilotica, Achras zapota, Datura stramonium, Emblica officinalis, Eucalyptus globules, Lawsonia inermis, Mimusops elengi, Peltophorum pterocarpum, Polyalthia longifolia, Prosopis juliflora, Punica granatum and Sygigium cumini have recorded significant antifungal activity **(**.Mayuri *et al*. 2014;

Natarajan, and Lalithakumari,.1987; Satish *et al*. 2008) [2,16,17]. A study by Themnozhi, and Sivaraj, (2009), Mahesh and Satish [18, 19], using Polyalthia longifolia shows killing efficacy of multidrug resistance microbes including Candida albicans. There are reports available with regard to the antifungal activity of medicinal plants against *Candida albicans*.The minimum inhibitory concentrations (MIC) of clove, sweet flag and eugenol against *Candida albicans* were studied by Antrasen and Amalabatra., (2012),[1] and Mathur,*et al*.,(2001) [21]. *Euphorbia heterophylla Tamilnadia uliginosa* and *Capparis sepiaria* plant derivatives showed *in vitro* antifungal potential against strains of *Candida albicans* (Ali-Shtayeh *et al*., 1999; Grayer, and Harborne, 1994) [3, 15].

4. Conclusion

In conclusion, the result obtained in this study clearly demonstrate broad spectrum antifungal activity of *Padina gymnosphora* whole plant extract against the nosocomial fungus, Candida albicans *Aspergillus niger*. 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 aqueous extract.

6. Acknowledgement

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