ORIGINAL ARTICLE

# Anticandidal activity of *Diospyros melanoxylon* Roxb. Bark from Similipal Biosphere Reserve, Orissa, India

**S. K. Panda, D. Dubey, S. K. Dutta**
Post Graduate Department of Zoology, North Orissa University, Baripada-757 003, India

The anticandidal activity and phytochemicals of bark of *Diospyros melanoxylon* Roxb. (*Ebenaceae*) was evaluated against four species of *Candida* viz. *C. albicans, C. krusei, C. parapsilosis* and *C. tropicalis.* Both polar and nonpolar extracts viz. petroleum ether, ethyl acetate, ethanol, methanol and aqueous were prepared and studied for anticandidal activity using agar cup and broth dilution methods. Although all five extracts showed promising anticandidal activity against all tested *Candida* species, yet maximum activity was observed in ethanol extract. Minimum inhibitory concentration values for most of the extracts ranged from 0.375 to 6.0 mg/ml, while the least minimum fungicidal concentration value was observed at 3.0-6.0 mg/ml. Phytochemical analysis exhibited the presence of carbohydrates, protein and amino acids, tannin and phenolic compound, glycoside, steroid and sterols and flavonoids in different extracts. Thin layer chromatography bio-autography showed zones of inhibition 22 mm (*C. parapsilosis*) and 21 mm (*C. tropicalis*) with Rf value 0.72 for ethanol extract. These results exhibit the anticandidal activity of *D. melanoxylon* bark extracts, which may be useful in treatment of candidiasis. However, the active components responsible for antifungal activity need to be evaluated.

**Key words:** Candidiasis, *Diospyros melanoxylon*, MIC, plant extract, Similipal Biosphere Reserve.

## INTRODUCTION

Fungi are the fifth most common pathogens, after *Enterobacteriaceae, Staphylococcus aureus, Pseudomonas aeruginosa* and coagulase-negative *Staphylococci*[1] Over the past few years, yeast, from the genus *Candida*, continued to be among the important etiological agents of nosocomial infection. Systemic candidiasis in hospitalized patients has increased steadily over the past four decades and represents a significant cause of morbidity and mortality among severely ill individuals.[2-4] A nationwide increase of 1.8 fungal infections per 1000 discharges was reported from 1980 to 1990, and 86% were due to *Candida* species.[5] Candidemia is the fourth most prevalent cause of bloodstream infections,[5,6] although its attributable mortality (40%) exceeds that of bacteremia.[6] This increase in fungal infections is exacerbated by the increasing population of immunocompromised patients, the prevalence of treatment with multiple broad-spectrum antibiotics, and the common use of indwelling intravascular devices.[6,7] *C. albicans* used to be the most common pathogen, but in recent years there has been a major shift toward other *Candida* types such as *C. tropicalis, C. krusei, C. glabrata* and *C. parapsilosis*.[2] Autopsy data indicate that more than half of the patients who die with malignancies are infected with *Candida* species.[8] Fungal resistance has received little attention in contrast to the critical importance of bacterial resistance. High degrees of antifungal drug resistance have been reported in *Candida* species and these have exhibited primary resistance patterns toward nystatin, clotrimazole, fluconazole, itraconazole and amphotericin B.[9,10]

The Similipal massif lies between 21°-28′ and 22°-08′ North latitude and 86°-04′ and 86°-37′ East longitude in the Mayurbhanj district of Orissa, covering 5569 km$^2$ of forest land, a unique habitat of mixed tropical forest, which harbor varied flora and fauna. The Similipal Biosphere Reserve[11] has been divided into three zones: (i) core zone (ii) buffer zone and (ii) transition zone [Figure 1], which are enriched with a variety of medicinal plants. The total number of species comprising the flora of the hills is 990, representing 145 families of vascular plants, with occurrence of more than 500 medicinal plants.[12,13] However, limited studies have been carried out on medicinal plants in general and antimicrobial activity of phytochemicals from Similipal Biosphere Reserve.[11,14-15] Mallavadhani *et al*[16] found the antimicrobial activity of *Diospyros melanoxylon* leaves, by isolating large quantities of amyrins (0.94%) and ursolic acid (0.56%), along with their synthesized lipophilic fatty acid ester chains against Gram-positive and Gram-negative bacteria. Rath *et al*[14] found the antimicrobial activity of *D. melanoxylon* from Similipal Biosphere Reserve against eight human pathogenic bacteria and



**Address for correspondence:** Dr. Sushil K. Dutta, Post Graduate Department of Zoology, North Orissa University, Baripada -757 003, India.
E-mail: sk_dutta@yahoo.com
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two species of *Aspergillus*. No reports are present so far regarding the anticandidal activity. This encouraged us to evaluate the anticandidal activity of Indian medicinal plant, *D. melanoxylon* Roxb. (*Ebenaceae*) bark, particularly against *C. albicans, C. krusei, C. parapsilosis* and *C. tropicalis*. Selection of the medicinal plant for the present study was based on its ethnomedicinal usages and preliminary screening of antimicrobial activity made by the authors.[15,16] The tribes of Similipal applied bark paste externally to cure scabies, itches and allied skin infections. A mixture of decoction of young leaves (3 ml) with unboiled egg (2 gm) and rice bran oil (2 ml) is prescribed for the treatment of night blindness.

## MATERIALS AND METHODS

### Plant Material

Bark and leaves of *D. melanoxylon* were collected in the month of April, 2007, from Similipal Biosphere Reserve, Mayurbhanj, Orissa. The collected bark and leaves along with complete herbarium of the plant of *D. melanoxylon* was sent for identification and finally was authenticated by Dr. A. K. Biswal, Department of Botany, North Orissa University, Baripada, and a voucher specimen (No. 140) was deposited in the Department of Botany, North Orissa University. The shed dried healthy bark was powdered separately using mechanical grinder and then were passed through sieve so that uniform powder size is maintained.

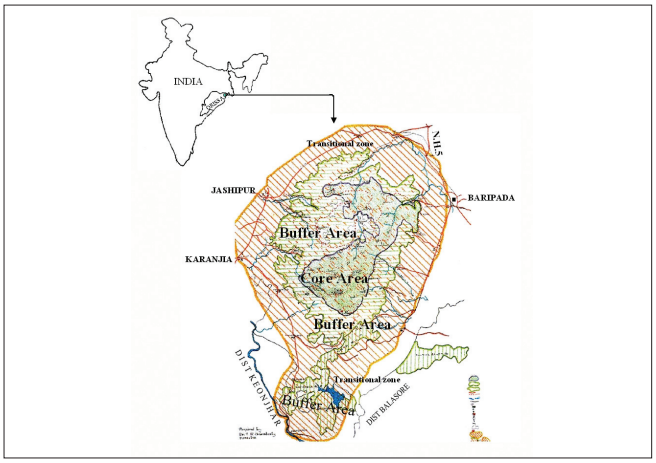


**Figure 1:** Map of Similipal Biosphere Reserve located on northern part of Orissa

### Preparation of Extract

One hundred gram of powdered plant material was taken in five separate conical flasks and soaked with 200 ml of each solvent (petroleum ether, ethyl acetate, ethanol, methanol and distilled water) at room temperature for 48 h. The extract was filtered through Buchner funnel using Whatman no. 1 filter paper. The filtrate was evaporated to dryness under reduced pressure and the concentrated extracts were freeze-dried to remove the solvent at -2°C till further use. The yield of each extract was calculated and stored for further use.

### Microorganisms and Growth Media

Four species of *Candida* viz. *C. albicans, C. krusei, C. parapsilosis* and *C. tropicalis* are used as the test organisms and are obtained from SCB Medical College, Cuttack, India [Table 1]. These organisms were cultured on Sabouraud dextrose agar at 31°C for 48 h and the stock culture was maintained at 4°C and subcultured as needed.

### Phytochemical Analysis

Qualitative phytochemical analysis was carried out using method described by Trease and Evans.[17] Each extract was screened for presence of alkaloids with Mayer's, Wagner's, Hager's and Dragendorff's reagents; flavonoids (NaCl and HCl); carbohydrates with Molisch's, Benedict's and Fehling's reagent; glycosides with Keller-Kiliani and Borntrager's; protein and amino acids with Biuret, Xanthoprotein, Ninhydrin and Millon's reagent; tannin and phenolic compound ($FeCl_3$ and Gelatin); gum and mucilage (KOH); triterpenoid with thionylchloride; steroid and sterols with Liebermann Burchard and Salkowski's reagents; and vitamin C with indophenols and nitroprusside reagents.

### Assay for Anticandidal Activity by Agar Cup Method

The agar cup method of Barry[18] was followed with little modification to ensure the anticandidal activity of the extracts. Plates of Sabouraud dextrose agar media were seeded with a 100 µl ($1.0 \times 10^5$ CFU/ml) of suspension of actively growing overnight culture of yeast cells. Wells (6 mm diameter) were made on Sabouraud dextrose agar plate using a sterile standard cork borer. The bottoms of the wells were sealed by pouring 50-100 µl of molten Sabouraud dextrose agar into scooped out wells. A 50 µl

**Table 1: List of *Candida* species used to assess the antifungal activity**

| Organism | Relevant properties | | Sources |
|---|---|---|---|
| | Resistant to | Sensitive to | |
| *Candida albicans* | Ak, B, Ce, Nf | Ap, Cc, It, Kt, Ns, Pb | SCB Medical College, Cuttack, Orissa |
| *Candida krusei* | Ap, B, Cc, It, Kt, Ns | Ak, A, B, Ce, E, Gf, G, Nf, Pb | SCB Medical College, Cuttack, Orissa |
| *Candida parapsilosis* | Ak, B, Ce, Nf | Ap, Cc, It, Kt, Ns, Pb | SCB Medical College, Cuttack, Orissa |
| *Candida tropicalis* | Ak, B, It, Ce, Nf | Ap, Cc, Kt, Ns, Pb | SCB Medical College, Cuttack, Orissa |

Amikacin-Ak (30 µg); Amphotericin- Ap (100 unit); Bacitracin- B (10 units); Cephotaxime- Ce (30 µg); Ciprofloxacin- C (10 µg); Clotrimazole- Cc (10 µg); Erythromycin- E (15 µg); Gentamycin - G (10 µg); Gatifloxacin- Gf (30 µg); Itraconozole- It (10 µg); Ketoconazole- Kt (10 µg); Nitroflurotoin- Nf (300 µg); Nalidaxic- Ns (100 µg); Polymyxin B- Pb (300 unit).

(10 mg/ml in DMSO) of extract were poured into the wells and allowed to evaporate water. The yeast seeded plates were incubated at 31°C for 48 h, after which the diameters of zone of inhibitions were measured. Each experiment was carried out in triplicates. The average diameter of the inhibition zone was taken for evaluating the anticandidal activity of the extracts.

**Evaluation of MIC and MFC**

A twofold broth microdilution method was adopted to determine MIC using 96-well microtiter plate and tetrazolium salt, 2,3,5-triphenyltetrazolium chloride (TTC) with slight modification of the method described by Eloff[19] for *Candida* species. In the plate, A1 to H1 consisted of SDB and test organism while A2 to H2 consisted of SDB with 20µl of DMSO and test organism. A3 to H3 were having the stock solution (30 mg/ml) of the test extracts and A4 to H4 till A9 to H9 were the wells in which the test extracts were serially diluted using Sabouraud dextrose broth. Wells A12 to D12 were control having 20 µl of DMSO and E12 to H12 served as control over control. All wells were dispensed with 100 µl of SDB. About 100 µl of the herbal extract was transferred from stock test solution to the first well, that is, from A4 to H4 containing 100 µl of SDB. About 100 µl of the SDB containing herbal extract was then transferred to the next well to create serial dilutions. An aliquot of 100 µl of the 0.5 McFarland–adjusted activated culture in Sabouraud dextrose broth was then added to all the wells except the blank. Five microliter of 0.5% TTC was further added to all the dilutions, blank, control and control over control. The final volume of all the wells was 205 µl. The Microplate was sealed and incubated at 31°C at 110 rpm. Each assay was repeated twice. An aliquot of 10 µl of the broth from each culture tube exhibiting MIC and control tubes were taken aseptically and were plated on 1-day-old Sabouraud dextrose agar plate as point inoculums and allowed to dry for 10 min under the laminar air hood to determine MFC. These were then sealed and incubated at 31°C for 48 h and observed for growth of the yeast.

**Thin Layer Chromatography Bio-autography**

The anticandidal activity of the crude and chromatographic fractions was ascertained by bio-autography using agar overlay method with minor modification of Runyoro *et al*.[20] Plant extract samples (5 µl) were spotted on 2.5 cm from the base of the Thin layer chromatography (TLC) plate and developed in the solvent system that separated the compound most precisely. After drying overnight culture of test organisms was centrifuged at 3000 rpm for 10 minutes and resuspended in sterile saline and added to a molten Sabouraud dextrose agar (HiMedia) containing 1% glycerol (v/v). This overlay was poured on TLC plate in a Petri plate and allowed to solidify under aseptic conditions. The plate was then incubated at 31°C for 48 h. The active compounds showed over the plates as white zones. The Rf value of the active compounds was recorded.

## RESULTS

Phytochemical screening of five extracts (petroleum ether, ethyl acetate, ethanol, methanol and aqueous) of bark has been summarized in Table 2. Percentage yield of different extracts were in the order ethanol > aqueous > methanol > ethyl acetate > petroleum ether. Evaluation of phytochemicals such as alkaloid, flavonoid, carbohydrates, glycosides, proteins and amino acids, steroid and sterols revealed the presence of most of constituents in polar extracts such as ethanol, methanol and aqueous extracts compared to nonpolar extracts (petroleum ether and ethyl acetate). However, steroid and sterol was found to be universally occurring in all the extracts.

Anticandidal activity of different extracts of bark of *D. melanoxylon* was evaluated against four *Candida* species by

**Table 2: Phytochemical screening of different extracts of *D. melanoxylon* bark**

| Phytochemical | Petroleum ether | Ethyl acetate | Ethanol | Methanol | Aqueous |
|---|---|---|---|---|---|
| Color | Brownish | Dark brown | Light brick red | Brownish | Brownish yellow |
| Physical appearance | Hard, powder | Hard powder | Sticky | Sticky | Sticky |
| % yield | 3.94 | 6.46 | 9.34 | 6.56 | 7.54 |
| Alkaloid | - | - | + | - | + |
| Carbohydrate | - | + | - | - | - |
| Glycosidase | | - | ++ | + | - |
| Proteins and amino acid | + | - | ++ | - | - |
| Tannin and phenolic compound | - | +++ | ++ | ++ | - |
| Gum and mucilage | - | - | - | - | - |
| Steroid and sterols | ++ | ++ | +++ | ++ | + |
| Triterpenoid | - | - | - | - | - |
| Flavonoid | - | - | + | - | - |
| Vitamin C | - | - | - | - | - |

(+++) Present in high amount; (++) Present in less amount; (+) Present in trace amount; (-) Absent

agar cup method [Table 3]. Two tested *Candida* species viz. *C. krusei, C. tropicalis* showing resistance to these common antibiotics such as nystatin, clotrimazole, itraconazole and amphotericin B. All five extracts viz. petroleum ether, ethyl acetate, ethanol, methanol and aqueous extracts showed good zone of inhibition by agar cup method against all tested *Candida* species.

TLC bio-autography was tested with ethanol extract against *C. tropicalis* and *C. parapsilosis*. The zone of inhibition was measured 22 mm for *C. tropicalis* and 21 mm for *C. parapsilosis* with Rf value 0.72 [Figure 2]. For bio-autography TLC plates were developed twice in chloroform: methanol (6.6:3.4) to facilitate better separation of the components. TLC bio-autography confirms that the ethanol extract contains many more compounds with antibacterial activities than crude extracts. The result of MIC showed that 20% of extracts were active in a concentration of 0.375 mg/ml, 50% of extracts were active in a concentration of 1.5 mg/ml, and 75% of extracts were active in a concentration of 3.0 mg/ml [Table 4]. However, at concentration 6.0 mg/ml, 100% inhibition was recorded. The result of MFC showed that at concentration 6.0 mg/ml, 45% of the tested *Candida* strains were killed while the remaining 55% was inhibited at the same concentration.

## DISCUSSION

Over the past few years, yeast belonging to the genus *Candida* continued to be among the important etiological agents of nosocomial infection. Approximately one-half of all inpatient *Candida* infections occur in surgical ICUs, reflecting the importance of the alimentary tract as a source of *Candida* infection. *Candida* outbreaks are serious concerns because of the ability of this pathogen to spread from patient to patient and caregiver to patient.[21] The attributable mortality of candidemia ranges from 40% to 60%,[22] which is higher than the attributable mortality of bacterial blood-borne infections.[23] Different *Candida* species have differing effects on mortality. Patients with hematologic and solid tumor malignancies have a lower mortality with fungemia due to *C. parapsilosis* as compared with *C. albicans*.[24] The noticeable increase in the frequency of infections caused by non-*albicans Candida* species and the appearance of candidal isolates resistant to both amphotericin B and the newer azoles represent two important alterations in the pattern of *Candida* infections. Heavy use of azoles has been linked to a shift toward non-albicans species of *Candida* in the ICU.[25,26] In a study from the European Organization for Research and Treatment of Cancer,[24] 31% of blood-borne *Candida* isolates represented breakthrough fungemia in the presence of antifungal therapy. Non-*albicans* species of

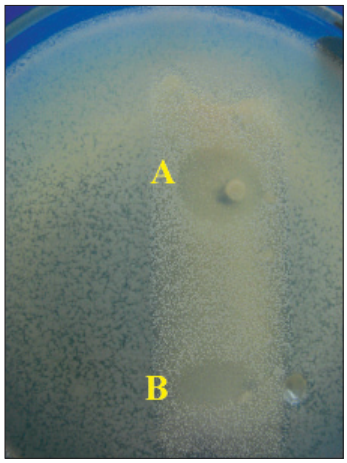


**Figure 2:** A. Zone of inhibition 22 mm (Rf 0.72) B. Origin TLC bio-autography against *Candia tropicalis*

**Table 3: Screening of anticandidal activity of *D. melanoxylon* bark by agar cup method**

| Strain | Zone of inhibition (in mm) of different extracts | | | | | |
|---|---|---|---|---|---|---|
| | Petroleum ether | Ethyl acetate | Ethanol | Methanol | Aqueous | Clotrimazole |
| *Candida albicans* | - | 14.0±1.00 | 16.7±1.53 | - | - | 20.0±1.00 |
| *Candida krusei* | 14.0±1.00 | 14.3±1.15 | 15.3±1.15 | - | 16.0±1.73 | * 14.3±1.15 |
| *Candida parapsilosis* | 12.3±2.08 | 16.7±1.53 | 19.3±2.08 | 16.0±1.73 | 14.0±1.00 | 28.7±0.58 |
| *Candida tropicalis* | 13.6±1.52 | 15.7±1.53 | 18.00±1.00 | 16.3±0.5 | 12.7±0.58 | 29.7±2.08 |

(Zone of inhibition of mean ± SD in mm); (-) No zone of inhibition; Zone of inhibition including 6 mm borer; *Cephotaxime

**Table 4: Screening of anticandidal activity by MIC and MFC**

| Strain | Petroleum Ether (mg/ml) | | Ethyl acetate (mg/ml) | | Ethanol (mg/ml) | | Methanol (mg/ml) | | Aqueous (mg/ml) | | Clotrimazole (mg/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC |
| *Candida albicans* | 6.0 | <6.0 | 3.0 | <6.0 | 6.0 | <6.0 | 6.0 | <6.0 | 6.0 | <6.0 | 0.002 | 0.002 |
| *Candida krusei* | 3.0 | <6.0 | 1.5 | <6.0 | 3.0 | 6.0 | 6.0 | <6.0 | 3.0 | 6.0 | *0.001 | *0.001 |
| *Candida parapsilosis* | 6.0 | <6.0 | 0.375 | 3.0 | 0.375 | 3.0 | 1.5 | 6.0 | 3.0 | 6.0 | 0.002 | 0.002 |
| *Candida tropicalis* | 3.0 | <6.0 | 0.375 | 3.0 | 0.375 | 3.0 | 1.5 | <6.0 | 3.0 | <6.0 | 0.002 | 0.004 |

***Cephotaxime**

*Candida* were isolated in 65% of breakthrough fungemias. These observations suggest that antifungal agents, especially when used as prophylaxis, may select for emergence of non-*albicans* species viz. *C. glabrata*, *C. parapsilosis, C. tropicalis*, and *C. krusei* promote drug-resistant strains. Development of drug-resistant pathogens demand new strategies and the native people's ethnobotanical knowledge, which has received less emphasis, is a valuable resource which should be utilized to advance health-oriented objectives. Since the incidence of *Candida* strains with multiple antibiotic resistances is increasing worldwide, it is of great importance to find effective treatments for infection of these pathogens. Novel, safe and effective compounds may be found through consultation with traditional healers or tribal peoples using herbal medicines. Certainly, indigenous plants are reservoirs of novel antimicrobials; they would play important roles in providing us with such bioactive in future. This encouraged us to evaluate the natural resources of our country to identify an antifungal agent, particularly against *C. albicans, C. tropicalis*, *C. parapsilosis* and *C. krusei.*

From Table 3 it is clear that zones of inhibition were obtained as maximum for ethanol extract followed by methanol, ethyl acetate, aqueous and petroleum ether extract. Among the yeast strain *C. tropicalis* (18 mm) and *C. parapsilosis* (19 mm) are most sensitive to all the extracts showed highest zone of inhibition followed by *C. krusei* (16 mm) and *C. albicans* (16 mm). However, *C. albicans* did not show any zone of inhibition against petroleum ether, methanol and aqueous extract. Carbohydrate and proteins and amino acids are universally present in all extracts. Hejgaard *et al*[27] showed proteins isolated from barley grain inhibit the growth of *C. albicans* in microtiter plate assay. Rauha *et al*[28] investigated that the presence of phenolic compounds in Finnish plant extracts and are effective in inhibiting the growth of the organisms, particularly *C. albicans*. Tannin and phenolic compound were present in three extracts viz. ethyl acetate, ethanol and methanol. Favell *et al*[29] isolated steroidal glycosides, namely, alexin, from crude extract of *Yucca gloriosa* L. and observed that alexin had a broad spectrum of antifungal activity. Hence, presence of these phytochemicals viz. proteins and amino acids, tannin and phenolic compound, glycoside, sterol and steroids in ethanol extracts may be responsible for anticandidal activity against all tested strains, which need further investigation. Presence of alkaloid on aqueous and ethanol extract may be the cause of better activity compared with other three extracts.

There is a need to exploit maximum potential in the field of medicinal and pharmaceutical sciences for novel and fruitful application, because herbal drugs are holistic gift of nature. Hence, *D. melanoxylon* bark extract could be utilized in developing more effective antimicrobial drugs in the management of nosocomial infections caused by *C. albicans, C. tropicalis, C. parapsilosis,* and *C. krusei*. Similipal possesses a rich tradition in the use of medicinal plants and an outstanding floral diversity of vascular plants; however, little research has been done on phytochemicals that can be used for therapeutic purposes. The present study has clearly demonstrated that the medicinal knowledge held by the tribal people is relatively measurable in a laboratory-based assay.

## REFERENCES


1. Vincent JL, Anaissie E, Bruining H, Demajo W, El-Ebiary M, Haber J, *et al*. Epidemiology, diagnosis and treatment of systemic *Candida* infection in surgical patients under intensive care. Int Care Med 1998;24:206-16.
2. Pfaller MA. *Nosocomial candidiasis*: Emerging species, reservoirs, and modes of transmission. Clin Infect Dis 1996;22:589-94.
3. Pfaller MA, Lockhart SR, Pujol C, Swails-Wenger JA, Messer SA, Edmond MB, *et al*. Hospital specificity, region specificity, and fluconazole resistance of *Candida albicans* bloodstream isolates. J Clin Microbiol 1998;36:1518-29.
4. Pfaller MA, Messer SA, Hollis RJ, Jones RN, Doern GV, Brandt ME, *et al*. Trends in species distribution and susceptibility to fluconazole among blood stream isolates of *Candida* species in the United States. Diagn Microbiol Infect Dis 1999;33:217-22.
5. Beck-Sague C, Jarvis WR. Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980–1990: National Nosocomial Infections Surveillance System. J Infect Dis 1993;167:1247-51.
6. Pittet D, Wenzel RP. Nosocomial bloodstream infections. Secular trends in rates, mortality, and contribution to total hospital deaths. Arch Intern Med 1995;155:1177-84.
7. Jarvis WR. Epidemiology of nosocomial fungal infections, with emphasis on *Candida* species. Clin Infect Dis 1995;20:1526-30.
8. Richardson MD, Warnock DW. Fungal Infection: Diagnosis and Management. London: Blackwell Scientific Publications; 1993.
9. Goff DA, Koletar SL, Buesching WJ, Barnishan J, Fass RJ. Isolation of flucanozole-resistant *Candida albicans* from HIV-negative patients never treated with azoles. Clin Infect Dis 1995;20:77-83.
10. Nolte FS, Parkinson T, Falconer DJ. Isolation and charecterisation of flucanazole and amphoterecin B resistant *Candida albicans* from blood of two patients with leukemia. Antimicrob Agents Chemother 1997;41:196-9.
11. Panda SK, Thatoi HN, Dutta SK. Antibacterial activity and phytochemical screening of leaf and bark extracts of *Vitex negundo* L. from Similipal Biosphere Reserve, Orissa. J Med Plant Res 2009;3:294-300.
12. Saxena HO, Brahmam M. The flora of Similipahar (Similipal), Orissa. Regional Research Labarotory. Bhubaneswar: CSIR Publication; 1989.
13. Pandey AK, Rout SD. Medicinal Plants of Similipal Biosphere Reserve. In: Das AP editors. Perspectives of Plant Biodiversity. Dehra Dun: Bishen Singh Mahendra Pal Singh; 2002. p. 681-96.
14. Rath SK, Mohapatra N, Dubey D, Panda SK, Thatoi HN, Dutta SK. Antimicrobial activity of *Diospyros melanoxylon* bark from Similipal Biosphere Reserve, Orissa, India. Afr J Biotechnol 2009;8:1924-8.
15. Thatoi HN, Panda SK, Rath SK, Dutta SK. Antimicrobial activity and ethnomedicinal uses of some medicinal plants from Similipal Biosphere Reserve, Orissa. Asian J Plant Sci 2008;7:260-7.
16. Mallavadhani UV, Mahapatra A, Jamh K, Reddy PS. Antimicrobial activity of some pentacyclic triterpines and their synthesizes

3-O-lipophilic chains. Biol Pharm Bull 2004;27:1576-9.
17. Trease GE, Evans WC. Text book of Pharmocognosy. 3rd ed. London: Bailliere Tindal; 1989. p. 81-90, 268-98.
18. Barry AL. Procedure for testing antimicrobial Agents in Agar media. In: Lorin V, editors. Antibiotics in Laboratory Medicine. USA: Williams Wilkins Co. Baltimore; 1980. p. 1-23.
19. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of the plant extracts for bacteria. Planta Medica 1998;64:711-3.
20. Runyoro DK, Matee MI, Ngassapa OD, Joseph CC, Mbwambo ZH. Screening of Tanzanian medicinal plants for anticandidal activity. BMC Comp Alt Med 2006;6:11-21.
21. Edwards JE, Bodey GP, Bowden RA, Buchner T, de Pauw BE, Filler SG, *et al.* International conference for the development of a consensus on the management and prevention of severe candidal infections. Clin Infect Dis 1997;25:43-59.
22. Jarvis WR. Epidemiology of nosocomial fungal infections, with emphasis on *Candida* species. Clin Infect Dis 1995;20:1526-30.
23. Edmond MB, Wallace SE, McClish DK, Pfaller MA, Jones RN, Wenzel RP. Nosocomial bloodstream infections in United States hospitals: A three-year analysis. Clin Infect Dis 1999;29:239-44.
24. Viscoli C, Girmenia C, Marinus A, Collette L, Martino P, Vandercam B, *et al*. Candidemia in cancer patients: A prospective, multicenter surveillance study by the invasive fungal infection group (IFIG) of the European Organization for Research and Treatment of Cancer (EORTC). Clin Infect Dis 1999;28:1071-9.
25. Georgopapadakou NH, Walsh TJ. Antifungal agents: Chemotherapeutic targets and immunologic strategies. Antimicrob Agents Chemother 1996;40:279-91.
26. Kao AS, Brandt ME, Pruitt WR, Conn LA, Perkins BA, Stephens DS, *et al*. The epidemiology of candidemia in two United States cities: Results of a population-based active surveillance. Clin Infect Dis 1999;29:1164-70.
27. Hejgaard J, Jacobsen S, Svendsen IB. Two antifungal thaumatin-like proteins from Barley grain. FEBS Lett 1991;291:127-31.
28. Rauha JP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, *et al*. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol 2000;56:3-12.
29. Fevell A, Kemertelidze E, Benidze M, Fallague1 K, Regli P. Antifungal activity of steroidal glycosides from *Yucca gloriosa* L. Phytother Res 2005;19:158-61.

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