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Research Article

ANTICANDIDAL ACTIVITY OF *CROTON ROXBURGHII* BALAK

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ABSTRACT

The present study was undertaken to find the anticandidal activity of the crude extracts of bark and leaf of *Croton roxburghii* Balak. (*Euphorbiaceae*) against four species of *Candida* viz. *C. albicans, C. krusei, C. parapsilosis, C. tropicalis*. Both polar and non-polar extracts viz. petroleum ether, acetone, ethanol, methanol and aqueous solution were prepared and studied for antifungal activity using agar cup and broth dilution methods. All test extracts such as petroleum ether, acetone, ethanol, methanol and aqueous showed zone of inhibition by agar cup method against *Candida* species. Among the strains *C. parapsilosis* is most sensitive to all the extracts showed highest zone of inhibition followed by *C. krusei* and *C. albicans.* MIC result showed that at concentration 1.25 mg/ml, most of extracts shows inhibitory effect. However, lowest MIC was recorded for ethanol extracts against *C. parapsilosis* (0.312 mg/ml). Evaluation of phytochemicals such as tannin and phenolic compounds, flavonoids, carbohydrates, protein and amino acids, steroids and gum mucilages revealed presence of most of constitutes studied in polar extracts such as ethanol, methanol and aqueous as compared to non polar extracts (petroleum ether and acetone). These results, exhibit the antifungal activity of *Croton roxburghii* Balak. bark and leaf extracts in treatment of candidiasis.

Keywords: *Croton roxburghii*, Antifungal activity, Candidiasis

INTRODUCTION

Infectious diseases, particularly skin and mucosal infections, are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits. An important group of these skin pathogens are the fungi, among which dermatophytes and *Candida* spp., besides certain pathogenic bacteria are the most frequent[1,2]. Furthermore, in the last few years, the numbers of immunosupressed and immunocompromised patients, who frequently develop opportunistic systemic and superficial mycoses[3,4] such as candidiasis, dermato-mycosis, fungal infections etc., have increased dramatically[5,6,7]. HIV infection commonly causes significant dermatological problems. It is estimated that 90% of the HIV positive patients will suffer with a mucocutaneous disorder during the illness. It is also estimated that up to 30% of people with AIDS will suffer from three different dermatoses. A rash may even be the presenting feature of the underlying HIV infection. These rashes can often be clinically atypical and difficult to diagnose. On top of this many of the skin problems are resistant to standard treatments. This is mainly due to the non-availability of effective antifungal drugs for systemic fungal infections and toxicity of available drugs like Amphotericin-B[8,9]. Thus there is an increased need for the development of alternative antipathogenic substances. One possible approach is to screen local medicinal plants in search of suitable chemotherapeutic antibacterial and antifungal substances.

In India only five species of *Croton* are used in ethnomedicine for treatment of various diseases, disorders and ailments like antifertility, boils, bowel complaints, chicken pox, cholera, cold and cough, constipation, cuts and wounds, diarrhea, dysentery, eye diseases, epilepsy, fever, gastric disorders, insanity, jaundice, liver complaints, malaria, rheumatism, ringworms, scurvy, spasmolytic agent, snake bite, sprains, etc.[10]. Tribal people in India used various parts of *C. roxburghii* against snake poisoning and to treat infertility, fever and wounds[11]. The tribes of Similipal use the cold decoction of root in sore throat. 2-3 teaspoon decoction of leaf is given in dysentery. Paste of root-bark is heated and applied to boils for either subsiding or hastening suppuration. However no reports are available on its antimicrobial activity except Thatoi *et al.*[12].

MATERIALS AND METHODS

Collection and identification of plant material

The fresh bark and leaf of *Croton roxburghii* were collected in the month of Dec, 2007 from the Similipal Biosphere Reserve, Mayurbhanj, Orissa. The collected bark along with complete herbarium of the plant of *Croton roxburghii* was sent for identification and finally was authenticated by Department of Botany, North Orissa University, Baripada.

Processing of plant material and preparation of extracts

The bark and leaf of *Croton roxburghii* were shed dried and followed by drying in hot dry oven for 1 hour at low temperature. Then it was powdered by mechanical grinder. The powder was stored in a closed container for further use. The dried powder was then use for extraction with different solvents. The solvents used for extraction were petroleum ether, acetone, ethanol, methanol and distilled water. The method of extraction was done by using cold percolation (steeping) by soaking of the plant material in each solvent for 3 days.

Qualitative chemical tests

Both leaf and bark extracts of *Croton roxburghii* were subjected to different chemical tests described by Trease and Evans[13].

Anticandidal activity

The agar cup method was used to study the anticandidal activity of the extracts as described by Panda *et al.*[14]. Four spp. of *Candida*[14] are used for testing of anticandidal activity. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the anticandidal activity of the extracts.

Determination of minimum inhibitory concentration (MIC)

In the present experiment, extracts which showed positive result were further evaluated for determination of MIC. A broth micro-dilution technique was adopted using 96 well micro-titer plates and tetrazolium salt, 2,3,5-Triphenyltetrazolium Chloride (TTC) was carried out to determine the MIC following the methods as described by Panda *et al.*[14]. Selected extracts were serially diluted in the 96-well plate with overnight culture of microorganisms (0.5 McFarland) grown at 35 ºC to obtain final concentration of extracts ranged from 0.2 mg/ml to 5.0 mg/ml. The micro-plate was sealed and incubated at 35 ºC at 130 rpm and observed for growth of the microorganism.

RESULTS AND DISCUSSION

Phytochemical screening and yield of different extracts

Evaluation of phytochemicals such as tannin and phenolic compounds, flavonoids, carbohydrates, protein and amino acids, steroids and gum mucilages revealed presence of most of constitutes

studied in polar extracts such as ethanol, methanol and aqueous as compared to non polar extracts (petroleum ether and acetone). However, flavonoids were found to be universally occurring in all the extracts irrespective of plant part used while vitamin-C is universally occurring in all leaf extracts (Table-1). Presence of phytochemicals such as flavonoids, tannin and phenolic compounds are mainly associated with antimicrobial activity [15,16,17]. Many medicinal plants containing flavonoids are reported to have been widely used in traditional medicine for the treatment of human diseases. Natural flavonoids have been reported to exhibit a variety important biological activity; including antimicrobial properties[18,19]. Therefore presences of flavonoids show the extract have antimicrobial activity. Carbohydrates, protein and amino acids are universally present in all extracts. Rauha *et al.*[20] investigated that the presence of phenolic compounds in Finnish plant extracts are effective in inhibiting the growth of the organisms, particularly *C. albicans*. Tannin and phenolic compound were present in all extracts except petroleum ether. Fevell *et al.*[21] isolated steroidal glycosides, namely, alexin, from crude extract of *Yucca gloriosa* L. and observed that alexin had a broad spectrum of antifungal activity.

Table 1: Screening of phytochemicals of *Croton roxburghii* bark (B) and leaf (L)

Name of the phytochemicals	Qualitative test	Petroleum ether extract		Acetone extract		Ethanol extract		Methanol extract		Aqueous Extract	
		B	L	B	L	B	L	B	L	B	L
Alkaloids	Mayer's reagent	-	-	-	-	-	-	-	-	-	-
	Dragendroff's	-	-	-	-	-	-	-	-	-	-
	Hager's reagent	-	-	-	-	-	-	-	-	-	-
	Wagner's reagent	-	-	-	-	-	-	-	-	-	-
Carbohydrates	Molisch's test	+	+	+	+	+	+	+	+	+	+
	Fehling's test	+	+	+	+	+	+	+	+	+	+
	Benedict's test	-	-	-	-	-	-	-	-	-	-
Tannins and phenolic compounds	With Ferric chloride	-	-	+	+	+	+	+	+	+	+
	With lead acetate	-	-	+	+	+	+	-	+	-	+
	With gelatin solution	-	-	-	-	-	-	-	-	-	-
Glycosides	Keller-Killiani test	-	-	-	-	-	-	-	-	-	-
	Legal Test	-	-	-	-	-	-	-	-	-	-
	Borntrager's test	-	-	-	-	-	-	-	-	-	-
Proteins and amino acids	Biuret test	-	-	-	-	-	-	-	-	-	-
	Ninhydrin test	-	-	+	+	+	+	+	+	+	+
	Xanthoprotein test	-	-	-	-	-	-	-	-	-	-
	Millon's test	-	-	-	-	-	-	-	-	-	-
Gum & mucilages	Molisch's test	-	+	+	+	-	+	-	+	-	-
Flavonoids	With NaOH	+	+	+	+	+	+	+	+	+	+
	With H_2SO_4	-	-	-	-	-	-	-	-	-	-
	With Mg/HCl	-	-	-	-	-	-	-	-	-	-
Saponins	Honeycomb foam	-	-	-	-	-	-	-	-	-	-
	Foam test	-	-	-	-	-	-	-	-	-	-
Steroids and sterol	Salkowski's test	-	-	-	-	-	-	-	-	-	-
	Liberman Burchard	-	-	-	-	+	-	+	-	-	+
Triterpenoids	Thionylchloride test	-	-	-	-	-	+	+	+	-	-
Oils and fats	With filter paper	-	-	-	-	-	-	-	-	-	-
	With alkaline KOH	-	-	-	-	-	-	-	-	-	-
Vitamin C	With Indophenol's	-	-	-	-	-	-	-	-	-	-
	Sod. nitroprusside	-	+	-	+	-	+	-	+	-	+

(+) Present; (-) Absent

Screening of anticandidal activity

The susceptibilities of the various *Candida* spp. both the plant extracts and standard antifungal agents were determined by the agar cup method are given in Table-2. All test extracts such as petroleum ether, acetone, ethanol, methanol and aqueous showed zone of inhibition by agar cup method against all *Candida* species. Among the strains *C. parapsilosis* is most sensitive to all the extracts showed highest zone of inhibition followed by *C. krusei* and *C. albicans.* Acetone extract of both bark and leaf showed highest zone of inhibition against all test *Candida* species. It is noteworthy that most of the aqueous and methanol extracts of *Croton roxburghii* produced no outstanding activity against *Candida* spp. used in the test.

Table-2: Screening of anticandidal activity of *Croton roxburghii* bark and leaf by agar cup method

Strain used	Petroleum ether		Acetone		Ethanol		Methanol		Aqueous		Antibiotic	
	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Cc	Ce
C. albicans	11.3±0.57	13.6 ±1.5	14.0± 1.0	15.0 ±2.6	12.7±0.58	13.3±2.08	13.3±2.08	11.3±0.57	11.3±0.57	10.6±0.57	20.0±1.0	-
C. krusei	11.3±0.57	13.6 ±0.5	15.0 ±1.7	15.0± 2.0	10.0±2.0	11.7±1.53	12.3±0.58	11.3±0.57	11.6±0.57	9.6±1.52	-	14.33± 0.5
C. parapsilosis	13.0±1.00	11.3±3.21	13.0±0.00	15.6±0.57	14.0±1.0	15.0±1.0	11.3±1.58	13.0±1.00	13.6±0.57	12.0±0.00	28.6±1.1	-
C. tropicalis	13.3±0.57	10.0±1.73	14.3±0.57	13.6±0.57	11.7±1.53	13.0±1.73	11.7±1.53	13.3±0.57	13.6±0.57	10.6±2.3	29.6±1.1	-

All values are mean zone of inhibition ± SD (-); No zone of inhibition; Zone of inhibition including 6 mm borer; Cc-Clotrimazole, Ce-Cephotaxime; Extract concentration (25 mg/ml)

Table-3: Results of MIC and MFC of all extracts of *Croton roxburghii* bark and leaf against *Candida* species

Plant extract	Test organism	Bark				Leaf				Clotrimazole			Cephotaxime		
		MIC	MFC	MFC/ MIC	TA	MIC	MFC	MFC/ MIC	TA	MIC	MFC	MFC/ MIC	MIC	MFC	MFC/ MIC
Petroleum ether	*Candida albicans*	1.25	5.0	4.0	13.4	1.25	>5.0	-	19.7	0.002	0.002	1.0	-	-	-
	Candida krusei	1.25	>5.0	-	13.4	1.25	>5.0	-	19.7	-	-	-	0.001	0.001	1.0
	Candida parapsilosis	1.25	>5.0	-	13.4	1.25	>5.0	-	19.7	0.002	0.002	1.0	-	-	-
	Candida tropicalis	0.625	5.0	8.0	26.8	2.50	>5.0	-	9.9	0.002	0.002	1.0	-	-	-
	Candida albicans	1.25	5.0	4.0	60.25	0.625	>5.0	-	185.6	0.002	0.002	1.0	-	-	-
Acetone	*Candida krusei*	0.625	5.0	8.0	120.5	0.625	>5.0	-	185.6	-	-	-	0.001	0.001	1.0
	Candida parapsilosis	0.625	5.0	8.0	120.5	0.625	>5.0	-	185.6	0.002	0.002	1.0	-	-	-
	Candida tropicalis	0.625	5.0	8.0	120.5	0.625	>5.0	-	185.6	0.002	0.002	1.0	-	-	-
	Candida albicans	1.25	2.50	2.0	126.6	0.625	2.50	4.0	282	0.002	0.002	1.0	-	-	-
Ethanol	*Candida krusei*	0.625	2.50	4.0	253.2	0.625	2.50	4.0	282	-	-	-	0.001	0.001	1.0
	Candida parapsilosis	0.312	2.50	8.0	506.4	0.625	2.50	4.0	282	0.002	0.002	1.0	-	-	-
	Candida tropicalis	0.625	2.50	4.0	253.2	0.312	2.50	8.0	564.1	0.002	0.002	1.0	-	-	-
	Candida albicans	1.25	5.0	4.0	98.4	1.25	>5.0	-	115.2	0.002	0.002	1.0	-	-	-
Methanol	*Candida krusei*	0.625	5.0	8.0	196.8	2.50	>5.0	-	230.4	-	-	-	0.001	0.001	1.0
	Candida parapsilosis	1.25	5.0	4.0	98.4	1.25	5.0	4.0	115.2	0.002	0.002	1.0	-	-	-
	Candida tropicalis	0.625	5.0	8.0	196.8	0.625	5.0	8.0	230.4	0.002	0.002	1.0	-	-	-
	Candida albicans	2.50	>5.0	-	31.3	2.50	>5.0	-	23.6	0.002	0.002	1.0	-	-	-
Aqueous	*Candida krusei*	2.50	>5.0	-	31.3	2.50	>5.0	-	23.6	-	-	-	0.001	0.001	1.0
	Candida parapsilosis	2.50	>5.0	-	31.3	1.25	>5.0	-	23.6	0.002	0.002	1.0	-	-	-
	Candida tropicalis	1.25	5.0	4.0	62.6	1.25	>5.0	-	47.2	0.002	0.002	1.0	-	-	-

Extract stock concentration-25 mg/ml

MIC result shows that at concentration 1.25 mg/ml, most of extracts are active (Table-3). However, lowest MIC was recorded for ethanol extracts against *C. parapsilosis*. The MFC values were higher than the MIC values of the extracts against all *Candida* species. The lowest MICs exhibited by extracts with MFC values four or eight time of MIC, in corresponding microorganisms, highlighting their interesting antimicrobial potency. From these results, it can be observed that, most of test samples exerted a lethal effect on the test organisms. In addition to these MMC/MIC ratio lower than 4 was obtained with most of the samples, suggesting killing effects come to mind[22]. The use of plants to heal diseases, including infectious one, has been extensively applied by people. Data from the literature as well as our results reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated. Therefore, our results revealed the importance of plant extracts when associated with antibiotics, to control resistant microorganisms, which are becoming a threat to human health.

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[23]. The referable mortality of candidemia ranges from 40% to 60%[24], which is higher than the attributable mortality of blood-borne bacterial infections[25]. 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*[26]. The noticeable increase in the frequency of infections caused by non-*albicans Candida* species and the appearance of candidal isolates resistance 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[27,28]. In a study from the European Organization for Research and Treatment of Cancer 31% of blood-borne *Candida* isolates represented breakthrough fungemia in the presence of antifungal therapy[26]. Non-*albicans* species of *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.

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REFERENCES

1. Caceres A, Fletes L, Aguilar L, Ramirez O, Ligia F, Tareena AM. Plants used in guatemala for the treatment of gastrointestinal disorders: Confirmation of activity against *Enterobacteriaceae*. J Ethnopharmacol 1993;38 :31-38.
2. Desta B. Ethiopian traditional herbal drugs, Part II: Antimicrobial activity of 63 medicinal plants. J Ethnopharmacol 1993;39 :129-139.
3. Rahalison L, Hamburger M, Monod M, Frenk E, Hostettaman K. Antifungal tests in phytochemical investigations: comparison of bioautographic methods using phytopathogenic and human pathogenic fungi. Planta Med 1994;60 :41-44.
4. Li E, Clark AM, Hufford CD. Antifungal evaluation of Pseudolaric acid B, a major constituent of *Pseudolarix kaempferi*. J Nat Prod 1995;58 :57-67.
5. Odds FC. *Candida* and candidiasis. Baillere Tindal, London; 1988.
6. Ribbon JW. The pathogenic fungi and the pathogenic actinomycetes. In: Medical Mycology, W.B. Sanders Company, Philadelphia, London, Toronto; 1988.
7. Diamond RD. The growing problem of mycoses in patients infested with human immuno deficiency virus. Rev Infect Dis 1991;13 :480-486.
8. Maddux MS. Brarriere SL. A review of complications of amphotericin B therapy: recommendation for prevention and management. Drug Intellectual Clin Pharmacol 1980;14 :177-181.
9. Saral R. *Candida* and *Aspergillus* infection in immunocompromised patients: an overview. Rev Infect Dis 1991;13 :487-492.
10. Salatino A, Salatino MLF, Negri G. Traditional uses, chemistry and pharmacology of *Croton* species (Euphorbiaceae). J Braz Chem Soc 2007;18 :11-33.
11. Gupta M, Mazumder UK, Vamsi MLM, Sivakumar T, Kandar CC. Anti-steroidogenic activity of the two Indian medicinal plants in mice. J Ethnopharmacol 2004; 90 suppl 1:21-25.
12. 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 :260-267.
13. Trease GE, Evans WC. Pharmocognosy, Braillier Tiridel and Macmillan Publishers; 1989.
14. Panda SK, Dubey D, Dutta SK. Anticandidal activity of *Diospyros melanoxylon* Roxb. bark from Similipal Biosphere Reserve, Orissa, India. Int J Green Pharm 2010;2 :42-47.
15. Hussain HSN, Deeric YY. Plants in Kano Ethnomedicine: Screening for antimicrobial Activity and alkaloids. Int J Pharmaceutics 1991; 29 suppl 1:51-56.
16. Runyoro DKB, Matee MIN, Ngassapa OD, Joseph CC, Mbwambo ZH. Screening of Tanzanian medicinal plants for anticandidal activity. BMC Comp Alt Med 2006;6 :11-21.
17. Panda SK, Behera BC, Dutta SK. Anticandidal activity of *Vitex negundo* L.: An ethanomedicinal plant. J Pure Appl Microbiol 2009; 3 suppl 2:777-784.
18. Alcaraz lE, Blanco SE, Pulg ON. Antibacterial activity of flavonoids against methicillin resistant *Staphylococcus aureus* strains. J Theor Biol 2000;205 :231-240.
19. Budzianowski J, Korzeniowska K, Chamara E, Mrozikiewicz A. Microvascular protective activity of flavonoid glucuronides fraction from *Tulipa gesneriana*. Phytother Res 1999;13 :166-168.
20. Rauha JP, Remes S, Heinonen M, Hopia A, Kahkonen M, Kujala T, et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol 2000; 55 suppl 1:3-12.
21. Fevell A, Kemertelidze E, Benidze M, Fallague1 K, Regli P. Antifungal activity of steroidal glycosides from *Yucca gloriosa* L. Phytother Res 2005;19 :158-161.
22. Carbonnelle B, Denis F, Marmonier A, Pinon G, Vague R. Techniques usuelles. Bactériologie médicale. SIMED, South Africa; 1987.
23. 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.
24. Jarvis WR. Epidemiology of nosocomial fungal infections, with emphasis on *Candida* species. Clin Infect Dis 1995;20 :1526-1530.

25. Edmond MB, Wallace SE, McClish DA, Pfaller MA, Jones RN, Wenzel RP. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. Clin Infect Dis 1999;29 :239-244.
26. 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 :1071-1079.
27. Georgopapadakou NH, Walsh TJ. Antifungal agents: chemotherapeutic targets and immunologic strategies. Antimicrob Agents Chemother 1996;40 :279-291.
28. Conn LA., 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 :1164-1170.