

NATURAL DRUGS

ANTIMYCOTIC POTENTIAL OF *CRATAEVA RELIGIOSA* HOOK AND FORST
AGAINST SOME SELECTED FUNGAL PATHOGENSSABUJ SAHOO*, SAGAR K. MISHRA, PRASANA K. PANDA, SHYAMLENDU TRIPATHY,
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Abstract: *Crataeva religiosa* Hook and Forst belonging to family Cappariaceae (Cappaceae) was selected based on its ethnopharmacological uses like diuretic, laxative, lithonotriptic, antirheumatic, antiperiodic, bitter tonic, rubifacient and counterirritant and was investigated to evaluate *in vitro* antimycotic potential of petroleum ether, chloroform, ethanolic and aqueous extracts against *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Cryptococcus marinus* and *Aspergillus niger* by disc diffusion method. The minimum inhibitory concentrations of *C. religiosa* extracts were found in the range of 0.062 – 0.5 mg/disc. The ethanolic extract significantly inhibits the growth of selected fungal pathogens, whereas aqueous extract do not show zone of inhibition against the tested *Candida* species. The results indicate the possible therapeutic uses of the plant as a potent antifungal agent.

Keywords: *Crataeva religiosa* Hook and Forst, ethnopharmacological, antimycotic potential, disc diffusion

Crataeva religiosa Hook and Forst belonging to family Cappariaceae (Cappaceae) is a tree usually cultivated in the vicinity of the temples of central and eastern India (1 – 3). The plant is popularly known as Assmarighna and Pashuganda in Sanskrit, Three leaved caper in English and Barun in Hindi (4). The plant material was collected from the rural belt of Bhubaneswar, and was identified and authenticated in Regional Research Laboratory, Bhubaneswar, Orissa, India. The voucher specimen bearing no. 9995 was deposited at the herbarium of Regional Research Laboratory, Bhubaneswar.

C. religiosa bark contains saponins and sugars. The plant parts used for medicinal purpose include stem barks, leaves and root barks (2, 4). The plant is used ethnopharmacologically as diuretic, laxative, lithonotriptic, antirheumatic, antiperiodic, bitter tonic, rubifacient and counterirritant (2, 4, 5). In folklore the bark is specifically used in urinary disorders including kidney and bladder stones, antiemetic and calculous affection and as an antidote in snake bite (4, 5). The ethnopharmacological

information regarding its use against urinary disorders and pathological skin conditions prompted us to select some of the fungal pathogens including *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Cryptococcus marinus* and *Aspergillus niger*.

The present study is intended to screen the antimycotic activity of various extracts of *C. religiosa* bark against different test fungal pathogens and to search for a newer antifungal agent to treat opportunistic microbial infections (6). *C. religiosa* extracts showing zone of inhibition against test fungi were compared with that of the standard antifungal agents (7) including fluconazole, clotrimazole, amphotericin B and ketoconazole.

EXPERIMENTAL

After authentication the bark of the flowering plant was collected in bulk during February, washed under running tap water to remove soil and dirt particles and then shade dried and pulverized by mechanical grinder. The resulting coarse powder

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was extracted successively using Soxhlet apparatus with various solvents of increasing polarity, starting with petroleum ether (b.p. 60-80°C) followed by chloroform, ethanol (95%) and finally with water for 24 h (8, 9). The extracts were concentrated by distilling off the solvents under reduced pressure and kept inside desiccator. The yield was calculated separately for each extract with respect to the air dried weight of the plant material which was found to be 1.8, 2.7, 10.3 and 9.6 (% w/w) for petroleum ether, chloroform, ethanol and aqueous extracts, respectively.

Extracts of *C. religiosa* were solubilized in N,N-dimethylformamide (DMF) and diluted with distilled water to make final concentrations as per the requirement. The solvent (DMF) was previously tested for antifungal activity against all test fungi and found to have no antifungal activity. Standard microorganisms *C. albicans* (MTCC 3017), *A. niger* (MTCC 1344) and *C. marinus* (MTCC *1029) were procured from Institute of Microbial Technology and Gene Bank, Chandigarh, India. Pathological samples of *C. tropicalis* and *C. krusei* were obtained from Post Graduate Department of Microbiology, Orissa University of Agriculture and Technology, Bhubaneswar, India.

Antifungal activity was determined by disc diffusion method (10-13) exhibiting zone of inhibition and minimum inhibitory concentration (MIC) determined by twofold serial dilution method (14, 15) as indicated in Table 1 and 2, respectively. The dilutions were performed by dispensing 1 mL of Czapak Dox Broth (CDB, HiMedia Laboratories Ltd., Mumbai.) and 1 mL of each extract to give desired concentration of 2 mg/mL and then serially diluted twofolds to achieve concentration up to 0.0078 mg/mL. Then, 50 µL of freshly prepared inoculum were added to each set of test tubes containing dif-

ferent extracts with graded concentration. The tube containing 1 mL of CDB, 1 mL of solvent and 50 µL of inoculum without the extract was taken as control. The set of inoculated tubes with extracts at different concentrations along with control were treated for 24 h and the contents of each tube were thoroughly mixed.

To freshly prepared SDA plates (molten state), 20 µL of inoculum was added (allowed to set), sterile discs were loaded with extracts (10 µL) at different concentrations and incubated at 28 ± 2°C for 48 h.

RESULTS

C. religiosa was selected based on its ethnomedicinal importance in Indian traditional system of medicines and the various extracts of the bark were screened *in vitro* for their antifungal properties. The results revealed that ethanolic extract among all extracts of *C. religiosa* exhibited significant activity comparable to standard antifungal agents against all test fungal pathogens and their MICs were found to be 0.062 – 0.5 mg/mL by twofold serial dilution method. The MIC values of *C. religiosa* extracts against selected fungal pathogens are summarized in Table 1.

DISCUSSION AND CONCLUSIONS

Aqueous extract inhibits *C. marinus* and *A. niger* at MICs 1 and 0.5 mg/mL, respectively. Aqueous and petroleum ether extracts of *C. religiosa* which did not inhibit the growth of *C. albicans*, *C. tropicalis*, *C. krusei* and *C. marinus*, respectively, up to a concentration of 2 mg/mL were considered inactive. A perusal of Table 2 enumerate the comparative zone of inhibition of fungal pathogens exhibited by different extracts of *C. religiosa* each

Table 1. Antimycotic activity of *C. religiosa* extracts against selected fungal pathogens and MIC by twofold serial dilution assay.

Organisms	MIC of <i>C. religiosa</i> (mg/mL)			
	PE	CH	ET	AQ
<i>Candida albicans</i>	0.125	0.062	0.062	n.a
<i>Candida tropicalis</i>	0.250	0.250	0.062	n.a
<i>Candida krusei</i>	0.062	0.125	0.125	n.a
<i>Cryptococcus marinus</i>	n.a	2	0.5	1.0
<i>Aspergillus niger</i>	1.0	1.0	0.250	0.5

PE, CH, ET, AQ = petroleum ether, chloroform, ethanol and aqueous extracts of *C. religiosa*

MIC = Minimum inhibitory concentration.

n.a. = not active – can not inhibit growth up to a concentration of 2 mg/mL.

Table 2. Comparative antibiogram pattern of *C. religiosa* against selected fungal pathogens at MICs by disc diffusion method.

Organism	Zone of inhibition (in mm)*							
	PE	CH	ET	AQ	FU	CC	AP	K
<i>Candida albicans</i>	16.3 ± 0.2	13.1 ± 0.40	18.8 ± 0.60	-	13.2 ± 0.35	16.2 ± 0.05	27.3 ± 0.2	12.5 ± 0.26
<i>Candida tropicalis</i>	17.5 ± 0.41	17.0 ± 1.0	20.1 ± 0.9	-	23.1 ± 0.11	22.2 ± 0.2	15.0 ± 0.05	18.0 ± 0.32
<i>Candida krusei</i>	11.7 ± 0.64	15.5 ± 0.33	13.1 ± 0.36	-	10.2 ± 0.05	13.7 ± 0.15	11.3 ± 0.2	10.4 ± 0.03
<i>Cryptococcus marinus</i>	-	-	14.7 ± 0.71	12.3 ± 0.45	11.4 ± 0.2	12.8 ± 0.55	13.4 ± 0.9	12.0 ± 0.2
<i>Aspergillus niger</i>	11.0 ± 0.4	12.2 ± 0.7	16.8 ± 0.3	13.3 ± 0.36	9.2 ± 0.65	22.1 ± 0.37	10.2 ± 0.65	9.8 ± 0.33

PE, CH, Et and AQ = petroleum ether, chloroform, ethanol and aqueous extracts of *C. religiosa*.

FU = Fluconazole (0.01 mg/disc), CC = Clotrimazole (0.01 mg/disc), AP = Amphotericin B (100 units/disc), K = Ketoconazole (0.01 mg/disc)

(-) = no zone of inhibition. * All the values are the mean ± standard deviation of three determinations.

tested at its respective MIC value with the potent standard antifungal agent by disc diffusion method.

The ethanolic followed by chloroform extracts showed better effect against the test fungal pathogens thus indicating the presence of higher contents of active phytoconstituents having antifungal potential. Further studies will aim at isolation and characterization of active phytoconstituents with antifungal potential.

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