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Short Communication

ANTIMICROBIAL EFFICACY OF THE ETHNOMEDICINAL PLANT SPECIES, *CANARIUM STRICTUM* ROXB

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ABSTRACT

Objective: This study is aimed to evaluate the antimicrobial activity of alcoholic leaf extracts of the ethno medicinal plant species, *Canarium strictum* belongs to the family, Burseraceae used by Irula tribal community of Walayar valley, the Western Ghats, Tamil Nadu, India.

Methods: Leaf samples were extracted with petroleum ether, ethyl acetate, and methanol. Antimicrobial activity against 10 bacterial strains and 10 fungal species was studied by using disc diffusion method, and the minimum inhibitory concentration was determined using macro-broth dilution method.

Results: All extracts showed a varied degree of antimicrobial activity against the tested pathogens. However, the methanol extract formed higher inhibition zone (16.41 mm) against the bacterium, *Moraxetta* sp. Methanol and the ethyl acetate extracts also showed a high degree of inhibition of the fungi, *Mucor rouxii* and *Rhizopus* sp. (20.67 and 15.72 mm respectively).

Conclusion: The methanolic extract was found to be more effective in inhibiting the colonial growth of the tested pathogens. Therefore, the results confirm the traditional knowledge of Irula tribals on the use of this species for curing the infectious diseases.

Keywords: *Canarium strictum*, Ethnomedicinal plant, Antimicrobial activity

Curing infectious diseases is difficult as the microbial pathogens develop resistance rapidly through mutation [1]. Therefore, investigation of less or no toxic, more potent and non anti-infective antibiotics is a challenging task [2]. However, plant derived drugs are more prominent and effective in curing such diseases due to the presence of a rich variety of bioactive compounds as secondary metabolites [3].

Traditional knowledge of our tribal communities, aboriginal people and local healers on medicinal plants for the treatment of infectious diseases is a more reliable source to identify the plant species for this purpose [4-6]. However, confirmation of traditional knowledge by scientific studies is remarkably incomplete, despite the availability of over 7000 medicinal species in India. In the present study, the traditional medicinal plant species, *C. strictum* which is prescribed by Irula tribal community of lower hills of Nilgiri Biosphere Reserve, the Western Ghats, India for curing infectious diseases was evaluated scientifically through antimicrobial studies.

Fresh leaf parts were collected from the population of *C. strictum* present in the Walayar valley of Western Ghats, Coimbatore District, Tamil Nadu and washed under running tap water, air dried and then homogenized to a fine powder and stored in air tight bottles.

250g air-dried leaf powder was subjected to 250 ml of methanol in soxhlet extraction for 8 h (50-85 °C). The extracts were concentrated to dryness in a flask evaporator under reduced pressure and controlled temperature (50-60 °C) to yield a crude residue. The crude residue was stored in the refrigerator. To obtain the other solvent extracts, the similar methods as used to obtain methanol extract was adopted by using the solvents viz., petroleum ether and ethyl acetate.

In vitro antimicrobial activity was evaluated for the chemical extracts of leaf part of the study plant, against ten bacterial species which include the gram-positive strains *viz*., *Micrococcus* sp., *Lactobacillus* sp., *Bacillus subtilis* and *B. thuringiensis* and gram-negative strains like *Pseudomonas aeruginosa, P. stutzeri, Escherichia coli, Klebsiella pneumoniae, Servatia* sp. and *Moraxetta* sp. and fungal species *viz., Aspergillus niger, A. flavus, A. baumannii, Fusarium oxysporum, F. solani, Mucor rouxii, Alternaria alternata, Candida albicans, Cladosporium* sp. and *Rhizopus* sp. All the microorganisms were maintained at 4 °C on nutrient agar slants (for bacteria) and PDA slants (for fungi).

The alcoholic extracts were tested for their effect against pathogenic bacteria and fungi by disc diffusion method [7]. Bacteria and fungi tested were inoculated into nutrient agar and PDA medium respectively. After an incubation period of 24 h at the temperature of 35 °C, three or four colonies isolated from these media were inoculated into 4 ml of nutrient broth and incubated for 2 h at 35 °C. The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing Muller-Hinton agar and PDA medium were streaked with these microbial suspensions of bacteria and fungi respectively. Disks of 6 mm diameter were impregnated with different extracts *viz.,* petroleum ether, methanol and ethyl acetate. Tetracycline is used as positive control. After equilibrium at 4 °C, the plates were incubated overnight at 37 °C and the diameter of any resulting zones of inhibition was measured. Each experiment was repeated at least three times.

The MIC was determined for antibacterial activities by the macro broth dilution methods [8, 9] at various concentrations (50, 100, 200, 400, 800 and 1000 µg/ml). The preparation of sterile broth inoculated with 1 ml of 10^6 CFU of bacteria uniformly. The nutrient agar medium supplemented with various concentrations of *C. strictum* extracts and 1 ml of respective test organism was inoculated and incubated at 37 °C for 24 h. After incubation, the number of colonies formed was counted. Controls were maintained by adding the same quantity of solvent without plant sample (negative control) and test culture with the standard, tetracycline (positive control). Triplicates were maintained for all experiments.

For antifungal activity, the samples were evaluated through the quantitative method described by Hirasawa *et al.* [10]. MIC assay was carried out by determining the percent inhibition of mycelial growth (PIMG) by the crude alcoholic extracts of *Canarium strictum***.** The crude extracts were added to PDA medium at the concentrations of 1250, 1500, 1750 and 2000 µg/ml separately. Agar discs (5 mm) were taken from 10 d old cultures of the above mentioned four human pathogenic

fungi and placed in the center of the Petri plates separately. For controls, the same size of agar discs of four fungi was placed in the same way on a fresh PDA plate separately which served as positive control. The negative control was maintained by adding the same quantity of solvent in the test culture and incubated at 25 °C for 3 d. Inhibitory activities were assessed by measuring the growth of mycelium on the treated media. The diameter of the colony (mm) was measured after the third day and documented. Triplicates were maintained and represented as mean±SD (Standard Deviation).

The results obtained in the present study revealed that the leaf extract of *C. strictum* exhibited variation in antibacterial activity against the tested bacteria and fungi (table 1). The inhibitory zones observed against the bacterium, *Bacillus thuringiensis* developed in petroleum ether extract (13.17 mm) and against *E. coli* in ethyl acetate extract (15.77 mm) were considerably higher than the other solvent extracts. Similarly, the inhibitory zone developed by the methanol extract against the gram negative bacterium, *Moraxetta* sp. was higher (16.41 mm). It may be explained that plant extract affects the bacterial cell membrane or interfered on essential bacterial enzymes like quinolones and sulfonamides and targeting protein synthesis [11, 12]. It was reported already that the antibacterial activity of alcoholic extracts of *Canarium strictum* analysed was notable against certain pathogenic bacteria [13]. It was observed further that the petroleum ether extract of this plant species showed inhibition activity only against certain specific bacterial strains. Different solvents have been reported to have different ability to drain phytoconstituents according to their solubility or polarity [14].

Table 1: Antibacterial activity of certain alcoholic leaf extracts of the species, *Canarium strictum*

Bacteria	Diameter of inhibition zone (mm)			
	Standard (Tetracycline)	Solvent extracts		
		Petroleum ether	Ethyl acetate	Methanol
Gram-positive bacteria				
Bacillus subtilis	7.13±0.56	-	13.15±0.67	-
B. thuringiensis	8.03±0.35	13.17±0.42	14.21±0.80	10.34±0.21
Micrococcus sp.	20.43±0.40	-	-	9.56±0.23
Lactobacillus sp.	21.03±0.45	8.07±0.55	9.03±0.44	7.41±0.59
Gram-negative bacteria				
Klebsiella pneumoniae	16.21±0.44	-	10.41±0.15	11.43±0.42
Escherichia coli	29.43±0.40	10.17±0.55	15.77±0.35	12.17±0.45
Pseudomonas stutzeri	12.73±0.46	-	7.16±0.72	8.32±0.25
P. aeruginosa	25.61±0.21	9.14±0.31	-	9.73±0.67
Serratia sp.	14.93±0.31	-	-	11.54±0.25
Moraxetta sp.	9.87±0.42	7.21±0.62	-	16.41±0.40

Experiments were performed in triplicates and represented as mean±standard deviation (SD).

Table 2: Antifungal activity of certain alcoholic leaf extracts of the species, *Canarium strictum*

Fungi	Diameter of inhibition zone (mm)			
	Standard (Tetracycline)	Solvent extracts Petroleum ether	Ethyl acetate	Methanol
Aspergillus niger	31.23±0.57	9.23±0.49	14.73±0.56	13.57±0.70
A. flavus	33.17±0.62	-	12.17±0.61	9.17±0.38
A. baumanii	34.63±0.32	-	10.46±0.23	10.83±0.80
Fusarium oxysporum	32.23±0.67	-	12.71±0.24	11.67±0.17
F. solani	24.73±0.14	.62±0.70	10.25±0.65	-
Mucor rouxii	27.63±0.21	16.73±0.67	20.67±0.61	14.70±0.62
Alternaria alternata	30.45±0.51	-	9.73±0.24	9.67±0.65
Candida albicans	15.76±0.15	-	10.72±0.82	8.27±0.55
Cladosporium sp.	13.67±0.16	-	-	7.07±0.91
Rhizopus sp.	35.32±0.51	10.16±0.32	12.17±0.57	15.72±0.13

Experiments were performed in triplicates and represented as mean±standard deviation (SD).

Table 3: The minimum inhibitory concentration (MIC) of methanolic leaf extract of *Canarium strictum* on certain pathogenic bacteria

Bacteria	Colony forming unit/ml at various extract concentrations (µg/ml)					
	50	100	200	400	800	1000
Bacillus subtilis	TNTC	TNTC	TNTC	85±0.82	-	-
B. thuringiensis	107±0.32	-	-	-	-	-
Escherichia coli	TNTC	TNTC	137±0.41	53±1.63	-	-
Serratia sp.	TNTC	38±0.65	15±0.82	10±0.41	-	-

TNTC–Too Numerous to Count, Experiments were performed in triplicates and represented as mean±standard deviation (SD).

Table 4: The minimum inhibitory concentration (MIC) of methanolic leaf extract of *Canarium strictum* on certain pathogenic fungi

Fungi	Zone of inhibition (mm) at various extract concentrations (µg/ml)			
	1250	1500	1750	2000
Rhizopus sp.	42±0.33	41±0.49	32±0.43	-
Fusarium solani	51±0.21	40±0.13	24±0.82	-
Aspergillus niger	34±0.82	20±1.62	18±0.16	-
Mucor rouxii	33±0.41	22±0.16	17±0.21	-

Experiments were performed in triplicates and represented as mean±standard deviation (SD).

The antifungal activity of various solvent extracts of leaf parts of the study species, *C. strictum* was also showed much variation against the fungal species tested (table 2). The ethyl acetate extract has the highest inhibitory activity (20.67 mm) against the fungus, *Mucor rouxii,* whereas the methanol extract exhibited higher inhibitory activity (15.72 mm) against another fungus, *Rhizopus* sp and the petroleum ether extracts registered highest inhibitory activity against *Mucor rouxii* (16.73 mm).

MIC was determined against the bacterial and fungal cultures. The level of inhibition of colonial growth of bacteria was observed to be varied according to the concentration of methanolic leaf extracts used (table 3). The methanolic leaf extract of *Canarium strictum* showed better activity against all the bacterial cultures tested at 1000 µg/ml except for *Bacillus thuringiensis* which has been inhibited even at 100 µg/ml by the extract. However, the two bacteria, *Escherichia coli* and *Serratia* sp. showed little susceptibility against the lower concentration of extracts used in the assay. As in bacteria, the inhibitory effect of methanolic leaf extract was also varied widely across the fungi tested (table 4). The methanolic leaf extract showed the notable level of inhibitory effect by 1750 µg/ml only when tested against fungi. Methanolic leaf extract showed a considerable inhibitory effect against the tested fungal cultures in the ascending series of *Rhizopus* sp., *Fusarium solani*, *Aspergillus niger* and *Mucor rouxii.*

The overall study on antimicrobial activity reports that the plant species contains many active compounds which by their synergistic effect may reduce or check the growth of microbial colonies [15]. The bioactive compounds isolated from the leaf part of this species *viz.*, triterpenoids like α-amyrin β-amyrin, β-amyrin acetate, (+) junenol, canarone, *epi*-khusinol and-Ψ-taraxasterol and epi-Ψ-taraxastane diol are reported to have an ameliorative effect on infectious diseases [16-18]. It can, therefore, be suggested that crude extracts contain potential antimicrobial compounds, and the obtained results may also be useful for evaluating substances of interest.

The more pronounced effective suppression of colonial growth of both pathogenic bacteria and fungi by the treatment of alcoholic leaf extract, the traditional knowledge of Irula tribal community of Walayar valley on the medicinal uses of the species *Canarium strictum*, particularly to heal infectious diseases like skin problems is confirmed. Therefore, after proper clinical trials, the species *C. strictum* can be used as a source of drug manufacture to treat skin diseases caused by specific pathogens.

CONFLICTS OF INTERESTS

Declare none

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