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Short Communication

Antimicrobial and Cytotoxic Activities of the Crude Extracts of *Lannea coromandelica*

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Abstract

The n-Hexane, chloroform and carbon tetrachloride extracts collected from the stem bark of *Lannea coromandelica* (Family-Anacardiaceae) were subjected to antimicrobial screening and brine shrimp lethality bioassay. All of the extracts showed poor inhibitory activity to microbial growth while the n-Hexane extract showed significant cytotoxicity having LC_{50} 4.135 µg/ml.

Keywords: *Lannea coromandelica*, Anacardiaceae, n-Hexane, chloroform, carbon tetrachloride, antimicrobial screening, brine shrimp lethality bioassay, cytotoxicity.

1. Introduction

Lannea coromandelica (Bengali name- Jiga, Jika, Jeol, Kafila etc.; Family- *Anacardiaceae*) is a medium-sized deciduous tree (bark thick, ashy-grey). The leaves are crowded at the end of branches (imparipinnate; 30-45cm long). Flowers are small, greenish yellow in compact fascicles of racemes at the end of the leafless branches. Drupes and reniform are produced in clusters from the end of leafless branches.[1,2,3] The bark is considered as astringent and stomachic, which is used as a lotion in impetigenous eruptions, leprous and obstinate ulcers. It also cures sprains, bruises, skin eruptions, heart diseases, dysentery and mouth sores. Decoction of the bark is used for toothache. Its bark along with the bark of *Aegle mermelos*, *Artocarpus heterophyllus* and *Sygygium cumini* is useful in impotency. Scrapped bark is chewed for 2-3 days to cure glossitis. Boiled leaves are applied as a fomentation for local swelling and pains.[4]

Previous phytochemical investigations of *Lannea coromandelica* reveals that flowing gum of the plant is a neutral polysaccharide composed of D-galactose and L-arabinose. Bark contains phlobatannins, β-sitosterol, physcion, physcion anthranol B, dl-epicatechin and leucocyanidin. Heartwood is rich in leucocyanidin. Flowers contain quercetin-3-arabinoside, ellagic acid, quercetin, isoquercetin and marin. Leaves contain leucocyanidin, leucodelphinidin, flavanoids, polyphenols, quercetin and rutin.[1,2]

2. Material and methods

Plant Materials: The plant *Lannea coromandelica* was collected from Norshingdi to carry out theantimicrobial and phytochemical studies. A voucher specimen has been deposited in the Bangladesh National Herbarium, Dhaka (DACB-35049) for identification.

Extraction and Isolation: The air-dried and powdered stem bark was subjected to cold extraction with methanol (ME) at room temperature with occasional shaking and stirring. The extract was then filtered through cotton plug and followed by Whatman No.1 filter paper. The volume of the filtrate was reduced using rotary evaporator at low temperature and pressure and afforded methanol extract. The concentrated methanol extract was subjected to solvent-solvent partitioning using the protocol designed by Kupchan and modified by Wagene[3]. The methanol extract was then successively extracted with *n*-hexane, carbon tetrachloride and chloroform of increasing polarity and afforded *n*-hexane (HE) carbon tetrachloride (CT) and chloroform (CF) soluble extracts.

Antimicrobial tests: The antimicrobial activity of the crude extracts was determined by the disc diffusion method[5] against thirteen bacteria (5 Gram positive and 8 Gram negative) and three fungi, collected from the stock cultures of the Institute of Nutrition and Food Science, University of Dhaka. The crude extracts (HE, CT and CF) were dissolved separately in chloroform and applied to sterile filter paper discs at a concentration of 400µg/disc and carefully dried to evaporate the residual solvent. Standard disc of Kanamycin (30µg/disc) and blank discs (impregnated with chloroform followed by evaporation) were used as positive and negative controls, respectively. The antimicrobial activity of the test samples was determined by measuring the diameter of zone of inhibition in millimeter and listed in Table-1.

Brine shrimp lethality test: Brine shrimp lethality bioassay technique of Meyer[6] was applied for the determination of cytotoxic property of the plant extracts of *Lannea coromandelica*. The crude hexane (HE), carbon

tetrachloride (CT) and chloroform (CF) soluble extracts were separately dissolved in DMSO. Four mg of each of the crude extracts (HE, CT and CF) was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.78125 μg/mL were obtained by serial dilution technique. Vincristine sulphate (VS) and DMSO were used as the positive and negative control, respectively. The median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration and the results are shown in Table-2.

3. Results and Discusion

Present investigation showed that the chloroform (CF) soluble fraction has the moderate sensitivity and carbon tetrachloride (CT) soluble fraction has only the mild sensitivity against almost all the bacteria and fungi, whereas hexane (HE) soluble fraction did not show any antimicrobial activity. The average zones of inhibition produced by CT and CF soluble fractions were found to be 6-9mm and 9-12mm, respectively at a concentration of 400μg/disc (Table-1). The CF fraction showed the strongest activity against *Bacillus subtilis, Vibrio mimicus* and *Vibrio parahemolyticus* having the zones of inhibition of 12mm for each. Besides this, other microorganisms were inhibited with a zone of 9-10mm. At the same time, CT fraction revealed only mild inhibitory activity against *Bacillus cereus, Bacillus megaterium, Staphylococcus aureus, Salmonella paratyphi, Salmonella typhi and Vibrio parahemolyticus* and the fungus *Sacharomyces cerevacae*. However, the bacteria, *Bacillus subtilis, Sarcina lutea, Escherichia coli, Pseudomonas aeruginosa, Shigella boydii, Vibrio mimicus and Shigella dysenteriae* and the fungus *Candida albicans & Aspergillus niger* were found to be resistant to it.

Following the procedure of Meyer[6], the lethality of the hexane (HE), carbon tetrachloride (CT) and chloroform (CF) fractions were evaluated on *A. salina*[6] after 24 hours of exposure the samples and vincristine sulphate (VS). The LC_{50} obtained from the best-fit line slope were 4.315, 7.434, 13.634 and 0.33 μg/ml for HE, CT, CF fractions and VS, respectively (Table-2). In comparison to positive control (VS), the cytotoxicity exhibited by *n*-hexane soluble fraction was promising and this clearly indicates the presence of antitumour or pesticidal compounds. On the other hand, carbon tetrachloride and chloroform soluble fractions demonstrated moderate citotoxicity, also indicate the presence of bioactive compounds in these fractions.

Table 1: Antimicrobial activity of the plant extracts of *Lannea coromandelica*

Test Microorganisms	Diameter of zone of inhibition (mm)			
	HE	CT	CF	KAN
Gram positive bacteria				
Bacillus cereus	-	6	9	39
Bacillus megaterium	-	6	10	32
Bacillus subtilis	-	-	12	20
Staphylococcus aureus	-	6	10	22
Sarcina lutea	-	-	9	20
Gram negative bacteria				
Escherichia coli	-	-	10	23
Pseudomonas aeruginosa	-	-	10	26
Salmonella paratyphi	-	7	10	30
Salmonella typhi	-	8	10	20
Shigella boydii	-	-	10	26
Shigella dysenteriae	-	-	10	24
Vibrio mimicus	-	-	12	24
Vibrio parahemolyticus	-	8	12	38
Fungi				
Candida albicans	-	-	9	24
Aspergillus Niger	-	-	10	32
Sacharomyces cerevacae	-	9	10	30

The diameter of zone of inhibition is expressed as mean ± SD (n=3); a diameter less than 6 mm was considered as inactive; HE: crude *n*-hexane extract; CT: crude carbon tetrachloride extract; CF: crude chloroform extract; KAN: kanamycin; "-" indicates no activity

Table 2: Brine shrimp lethality of the crude extracts of *Lannea coromandelica*

Sample	LC_{50} (μg/mL)
VS	0.33
HE	4.135
CT	7.434
CF	13.634

The values of LC_{50} are expressed as mean ± SD (n=3).
VS: vincristine sulphate (Std.)

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