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Research Article

Phytochemical and Pharmacological Investigations of *Lannea grandis* (Dennst.) Engl. Leaves Extracts

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ABSTRACT

Medicinal plants can serve as a prominent source of bioactive secondary metabolites which can exert beneficial effects to combat against many human diseases. *Lannea grandis* (Dennst.) Engl, a member of Anacardiaceae family has several applications in the ethnomedical practices. In this study, the ethanol extract of *L. grandis* leaf (ELG) along with its petroleum-ether and chloroform fractions were subjected to phytochemical analysis along with the evaluation of antimicrobial, anti-diarrheal, and sedative activities using standard protocols. Phytochemical screening was performed according to common phytochemical tests. Antimicrobial assay was carried out by disc diffusion method where the ethanol extract of *L. grandis* showed significant activity against tested bacterial species (zone of inhibitions = 10.8±0.85 to 13.8±1.84 mm). The same extract also exhibited the highest antifungal activity against *Blastomyces dermatitidis* (zone of inhibition = 20.5±0.35 mm). The chloroform fraction of *L. grandis* at the dose of 400 mg/kg body weight produced a significant ($p < 0.05$) anti-diarrheal effect with 40.69% inhibition of castor oil-induced diarrhea in mice. During sedative activity assay in mice, the plant extract at the dose of 400 mg/kg body weight produced mild reduction in the time of onset of sleep and increased the length of the sleeping time induced by a sedative, phenobarbitone. In conclusion, the plant *L. grandis* can be considered as a base for the development of new drugs and phytomedicine.

Keywords: *Lannea grandis*, phytochemical, antimicrobial, anti-diarrheal, sedative activity.

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INTRODUCTION

Since the time immemorial, people have been using herbal remedies and medicines to cure various diseases^{1,2}. The interest in drugs of plant origin is still increasing because plants are the valuable source of structurally diverse compounds, which possess therapeutic potential for treatment of human diseases². A lot of the clinically used therapeutic agents are of natural products origin. Thus, natural products have been well recognized and documented as a source of inspiration to drug discovery^{3,4}. Traditional medical practice is still the main vehicle of health care delivery today especially in the rural areas of the country where conventional medical facilities are not within the reach of most people⁵. Increase in the patronage of herbal medicines is likely to carry on because of the global economic recession. Moreover, a large proportion of the world population rely on herbal medicines for their medical care⁶.

Lannea grandis (Dennst.) Engl. (Family: Anacardiaceae), Synonym- *Lannea coromandelica* (Houtt.) Merr is a medium-sized deciduous tree which grows up to 10 to 20 m in height. The plant is widely distributed in Bangladesh, India and some other tropical countries⁷. Locally it is common as Bhadi, and is cultivated as a hedge plant in the roadside areas. The folk people in Bangladesh used the plant for various diseases including pain, inflammation and infection⁸. The leaf of the plant is reported to be useful in scurvy, skin diseases, dysentery, and pain. Leaves and barks were reported to have astringent properties and applied in toothache⁹. The ethanol bark extract of *L. coromandelica* exhibited significant pain relieving activity in mice model¹⁰. In an earlier report, polyflavonoid tannins were isolated from the stem bark of *L. coromandelica* which showed sporicidal activity¹¹.

Although the plant *L. grandis* is traditionally very important, but the scientific reports regarding its pharmacological studies are inadequate. Therefore, as a continuation of our

research on medicinal plants,¹²⁻¹⁴ the present study was designed to identify the phytochemicals as well as to determine the level of antimicrobial, anti-diarrheal, and sedative activity of leaves of *Lannea grandis* Engl.

MATERIALS AND METHODS

Collection and identification of plant material

Leaves of *L. grandis* were collected from the hill of Forest Research Institute, Chittagong, Bangladesh and were identified by Forest Research Institute, Chittagong, Bangladesh.

Extraction

The leaves were first air-dried followed by oven drying at 35°C and ground to coarse powder with a grinder. About 250 g of powdered sample was taken in an amber bottle and extracted with 97.7% ethanol for 7 days. Meanwhile another 150 g of same plant powder was subjected to hot extraction with 97.7% ethanol using Soxhlet apparatus¹⁵. The extract was filtered through a cotton plug followed by Whatman filter paper no.1. The volume of the filtrate was condensed at reduced temperature and pressure to obtain a gummy concentrate which is designated as ethanol extract of *L. grandis* (ELG). The resulting crude extract obtained by both cold and hot extraction was mixed together and was subjected to fractionation with petroleum-ether and chloroform subsequently to obtain petroleum-ether (PEF) and chloroform (CFF) soluble fractions, respectively. The amount of the ELG, PEF and CFF were 7.13 g, 4.56 g and 3.02 g, respectively.

Phytochemical screening

The ethanol crude extract of *L. grandis* and its petroleum-ether and chloroform fractions were examined by qualitative tests for the existence of the phytochemical constituents by using the standard methods^{16,17} as mentioned in Table 1.

Antimicrobial activity

The antimicrobial susceptibility of *L. grandis* was initially evaluated by the agar disc diffusion assay¹⁸ using a variety of test microorganisms (Table 2). Ciprofloxacin and fluconazole were used as standards. The organisms were obtained as pure culture from the Faculty of Biology, University of Chittagong, Bangladesh. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiments were carried out in triplicate and the results have been shown as mean \pm SD (Table 2).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the plant samples that revealed a good activity in disc diffusion assay were subjected to determine the MIC value by the serial tube dilution technique¹⁹ in broth medium, containing graded concentration of the plant extracts inoculated with the test organisms.

Test animals

Swiss Albino mice of either sex (aged 4-5 weeks) weighing 25-30 g were collected from the Animal Resources Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (icddr,b). They were kept in standard laboratory conditions at room temperature ($24 \pm 2^\circ\text{C}$) and relative humidity (60-70%) in a 12 h light/12 h dark cycle. All the experiments were conducted according to the protocol²⁰ approved by the institutional ethical committee.

Anti-diarrheal activity

The anti-diarrheal activity of *L. grandis* was evaluated by castor oil-induced diarrhea model in mice²¹. The experimental animals were arbitrarily divided into five groups having five mice in each. Negative control group received vehicle (1% Tween-80 in water) at a dose of 10 ml/kg whereas the standard group received loperamide at a dose of 3 mg/kg as oral suspension. Test groups received plant extracts (ELG, PEF and CFF) at the dose of 400 mg/kg orally. Thirty min later diarrhea was induced by oral administration of 0.4 ml castor oil to each mouse. During the observation period (4 h), the total latency period and the number of diarrheic feces excreted by the animals were recorded.

Sedative activity

The sedative activity of *L. grandis* was assessed by phenobarbitone sleeping time test in mice²². The test animals were randomly divided into five groups as mentioned above. The positive control group was given standard diazepam (1 mg/kg, i.p.) while the test groups received plant samples (ELG, PEF and CFF) orally at a dose of 400 mg/kg body weight. After 30 min of administration of test drugs, phenobarbitone at a dose of 40 mg/kg body weight was administered by intra-peritoneal route to all groups of mice to measure phenobarbitone induced sleeping time. The animals were observed for the latent period (time between phenobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex).

Statistical analysis

Data were expressed as mean \pm SD (standard deviation) and mean \pm SEM (standard error of mean). The results were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

The preliminary phytochemical screening study revealed that the crude ethanol extract, petroleum-ether fraction and chloroform fraction of *L. grandis* were rich of some vital chemical groups like alkaloids, steroids, tannins, saponins, glycosides and gummy types plants secondary metabolites (Table 1) which are responsible for exposing different types of pharmacological activity²³.

Table 1: Phytochemicals present in the ELG, PEF and CFF of *L. grandis*.

Examination	Name of the test	Plant sample		
		ELG	PEF	CFF
Reducing sugar	Fehling's solution test	+	-	-
	Benedict's test	+	+	+
Steroids	Salkowski & Libermann-burchared test	+	-	-
Glycosides	Salkowski & Libermann-burchared test	-	-	-
	Potassium dichromate test	+	-	+
Tannins	Keller-Kiliani test	-	-	+
	Ferric chloride Test	+	-	-
	Mayer's test	+	+	-
	Dragendorff's reagent test	+	-	-
Alkaloids	Wagner's reagent test	+	-	-
	Hager's reagent test	-	-	-
	Tannic acid test	-	-	-
Flavonoids	--	-	-	-
Saponins	--	+	-	+
Gums	--	-	-	+
Amides	--	-	-	-

[+ = presence; - = absent. ELG=Ethanol crude extract of *L. grandis* leaf, PEF =Petroleum ether fraction, CFF=Chloroform fraction of *L. grandis*]

The demand for novel antimicrobial agents from natural sources is mounting day by day²⁴. In order to find out the prospective antimicrobial principles, the plant *L. grandis* was subjected for preliminary antimicrobial screening by disc diffusion method. During antimicrobial test, the plant samples showed mild to moderate activity against tested organisms (Table 2). The ethanol extract showed antibacterial activity with zone of inhibition ranging from

10.8±0.85 to 13.8±1.84 mm. The maximum antibacterial potential was observed by ethanol extract of *L. grandis* against *S. aureus* (13.8±1.31 mm) and *E. coli* (13.8±1.84). The petroleum-ether fraction exhibited no activity against any test microorganisms while the chloroform fraction showed activity against *Shigella dysenteriae* (10.7±0.47) and *Escherichia coli* (11.83±1.03).

Table 2: Antimicrobial activity of different extract of *L. grandis*, ciprofloxacin and fluconazole:

Test organisms	Zone of inhibition (MZI±SD) mm		
	ELG	CFF	Standard
Gram positive bacteria			Ciprofloxacin
<i>Bacillus subtilis</i>	13±1.63	nd	28.2±0.85
<i>Staphylococcus aureus</i>	13.8±1.31	nd	28.5±1.087
Gram negative bacteria			
<i>Escherichia coli</i>	13.8±1.84	11.83±1.03	30.2±1.43
<i>Salmonella Typhi</i>	12±1.63	nd	30.7±2.57
<i>Shigella dysenteriae</i>	10.8±0.85	10.7±0.47	29.2±1.85
<i>S. sonnei</i>	10.8±1.03	nd	30.2±0.62
<i>Vibrio cholerae</i>	12.5±1.5	nd	27.7±2.1
Fungi			Fluconazole
<i>Blastomyces dermatitidis</i>	20.5±0.35	13.3±1.08	23.5±0.94
<i>Candida albicans</i>	nd	11.3±1.47	25.5±0.54
<i>Cryptococcus neoformans</i>	13.7±1.34	nd	23±2.16
<i>Microsporum spp.</i>	13.2±1.14	nd	25.3±1.78

*[nd: Not detected; MZI: Mean zone of inhibition (mm); SD = Standard deviation; Zone of inhibition under 8 mm was considered as less active and was discarded]

In case of antifungal screening, the ethanol extract of *L. grandis* showed strong antifungal activity against *B. dermatitidis* (zone of inhibition = 20.5±0.35 mm) while the chloroform fraction revealed moderate activity against *B. dermatitidis* (zone of inhibition = 13.3±1.08 mm) and *C. albicans* (zone of inhibition = 11.3±1.47 mm). During the MIC determination, the extracts displayed low MIC value of

15.625 µg/ml against *B. dermatitidis*. This result suggested the presence of antimicrobial compounds in the extractives. The manifestation of antimicrobial activity of *L. grandis* against both bacteria and fungi may be considered as a good source of bioactive compounds having antimicrobial properties²⁵.

Table 3: Anti-diarrheal effect of *L. grandis* extractives in mice

Treatment	MLP (h)	%MLP	TLP	% Inhibition of defecation	TNF (240 min)
Control (1% Tween 80)	0.81	100.0	4.05±6.36	0	86±3.049
Loperamide 3 mg/kg	1.40	172.84	7.00±7.17 ^a	62.79	32±1.48 ^a
ELG 400 mg/kg	1.07	132.09	5.35±3.82 ^b	5.81	81±2.33 ^a
PEF 400 mg/kg	0.94	116.05	4.70±6.17 ^a	19.77	69±2.72 ^b
CFF 400 mg/kg	0.87	107.41	4.35±4.22 ^a	40.69	51±2.08 ^a

*[^ap<0.05, ^bp<0.1; % MLP = (test group MLP×100)/Control MLP; TLP = Total latent period (MLP×5 ± SEM); TNF = Total number of feces (MD×5 ± SEM)]

Medicinal plants are gifted source of new anti-diarrheal drugs. There are a huge number of plant-based medicines that are reported to be helpful in treating diarrhea. Therefore, to find out a new anti-diarrheal agent from plant source, *L. grandis* was investigated for the evaluation of anti-diarrheal activity by castor oil-induced diarrhea in mice. Ricinoleic acid, the principal ingredient of castor oil is reported to bring out enhanced peristaltic movement and finally induces diarrhea²⁶. In the castor oil induced diarrheal test, the different extracts of the *L. grandis* at the doses of 400 mg/kg reduced the total number of diarrheal feces as well as increased the latency period in mice to a considerable extent and these effects are comparable to the control groups (Table 3). The chloroform fraction of *L. grandis* reduced the diarrheal episode by 40.69% inhibition. The standard loperamide produced 62.79% inhibition of diarrhea in mice. The ethanol extract, petroleum ether and chloroform fraction reduced the total number of feces to 81±2.33, 69±2.72 and 51±2.08, respectively. And the total latent periods were 5.35±3.82, 4.70±6.17, 4.35±4.22 h, respectively compared to the positive control (7.00±7.17 h) respectively. From the above experiment it should be claimed that the plant was found to be effective in the management of diarrhea. The anti-diarrheal effect of *L. grandis* might be caused by the bioactive phytoconstituents present in the test sample, however further studies are required²⁷.

Table 4: Sedative activity of *L. grandis* extractives in mice.

Treatment	TLP (min)	TST (min)
Diazepam 1 mg/kg	43±1.61 ^a	235±3.04 ^b
ELG 400 mg/kg	141±3.65 ^a	123±3.46 ^a
PEF 400 mg/kg	82±2.23 ^a	84±3.03 ^a
CFF 400 mg/kg	104±3.05 ^a	61±2.03 ^a

[* ^ap<0.01, ^bp<0.05, ^cp<0.1; TLP(Total latent period): (mean×5) ± SEM; TST (Total sleeping time): (mean×5) ± SEM; SEM = Standard Error of Mean]

The test samples (400 mg/kg body weight) moderately reduced the latency period as well as prolonged the phenobarbitone-induced sleeping time, with respect to the control (Table 4). These findings revealed that the plant

samples may have sleep inducing properties. The prolongation of sleeping time is probably through a CNS depressant action or a sedative action²⁸ of the test samples.

CONCLUSION

From these studies it was found that the crude extracts of the plant *L. grandis* contained important chemical groups like alkaloids, steroids, tannins, saponins, glycosides and gummy types. The plant also possesses significant antimicrobial, anti-diarrheal and sedative activity when compared with corresponding standard drugs. So, advance research is imperative to discover the bioactive principles and exact mechanisms of action.

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CONFLICT OF INTEREST

No competing interests.

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