

COMPARATIVE EFFECT OF *LANNEA COROMANDELICA* (HOUTT.) MERR. LEAVES AND STEM BARKS ON ACETIC ACID INDUCED PAIN MODEL IN MICE AND CHROMOGENIC REAGENTS: EXPLORING THE ANALGESIC POTENTIAL AND PHYTOCHEMICAL GROUPS

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Abstract

Lannea coromandelica (Houtt.) Merr (Anacardiaceae), a Bangladeshi medicinal plant, has long been used in indigenous medicine. The plant is reported to be used in the traditional medicine for all local swelling, pain and inflammation and in the treatment of tumour, ulcers, cancer, sprains, bruises, skin diseases, and dysentery. Both the leaves and the stem bark have painkilling and astringent property and so, mixed with toothpowder for use in toothache. The leaves are copped as fodder and fed the livestock in inflammatory and digestive problems. In this study, ethanol extracts of the leaves and stem bark of this traditionally valuable medicinal plant were screened for analgesic activity on acetic acid induced Swiss-albino mice- *Mus musculus* *in vivo* for the first time as well as studied for some important phytochemical groups. The dried extracts were dissolved in 99.8% ethanol and qualitatively analysed for bioactive chemical groups-alkaloids, glycosides, steroids, gums, reducing sugars, tannins, flavonoids, and saponins using standard chromogenic reagents. The colour intensity or the precipitate formation was used as analytical responses to these tests. Both the extracts were found to contain steroidal compounds, tannins, gums, reducing sugars, alkaloids as well as flavonoids as their major bioactive phytochemical groups. In analgesic activity test, the bark extract produced 92.92 % writhing inhibition and the leaf extract produced 95.14% writhing inhibition at the dose of 250 mg/kg of body weight compared to the standard diclofenac Na that inhibit 78.54% writhing inhibition at the dose of 25 mg/kg body weight by acetic acid induced writhing model in mice. Based on the results, it could be concluded that the ethanol extract of *Lannea coromandelica* (Houtt.) Merr leaves and bark possess significant analgesic activity and mood of action might involve a peripheral mechanism. The results rationalize its use in folkloric remedies especially against pain and inflammation.

Key words: *Lannea coromandelica*, Anacardiaceae, Analgesic activity, Phytochemical groups, *In vivo* acetic acid induced writhing test

Introduction

Numerous studies indicate that diets high in fresh leaves and plant parts are associated with a "healthy lifestyle". One reason for this is that these foods from the vegetative origin contain compounds called phytochemicals. Phytochemicals are compounds found in plants that have biochemical activities in the human body even though they have no nutritional value. The functions that phytochemicals perform in the human body include antioxidant activity, cytotoxic activity (in terms of cancer inhibition), cholesterol regulation and anti-inflammatory activity. Each plant part or vegetable is a unique package of phytochemicals, so consuming a wide variety of vegetarian diet provides the body with the broadest spectrum of benefits. In such a situation, many phytochemicals are consumed in small amounts. This approach is much safer than taking supplementary doses of particular phytochemicals, in larger doses, some phytochemicals are toxic [1].

Nature is the source of 87% drug used to treat all categorized human diseases. 25% of prescribed drugs are originated from plant. Till now about 80% people in developing countries rely on traditional plant based medicines for their primary health care. Focus on natural products is increasing day by day as it serves as an enormous source of new drugs of widespread indications. Natural products are vital in the treatment of pain, as a number of important analgesic agents have been derived from natural products, including plant-derived agents such as the aspirin, morphine, heroine. The painkiller drug market in Bangladesh is growing day by day every year due to an alarming rise of patients associated with pain. So, search for new painkiller drugs is the demand of time. Bangladesh has a rich and prestigious heritage of herbal medicines among the South Asian countries and lots of plants are used in its indigenous medicines. It is estimated that about 250 species of medicinal plants are used for the preparation of traditional medicines which is the half of total species of plants grown in Bangladesh. However, the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compound(s). Traditional resources and ecological diversity indicate that Bangladeshi plants represent an exciting resource for new drug discovery. As the people involved in indigenous medicine practice kept their secrets very much to themselves and newer synthetic drugs are becoming available; this system is gradually getting lost. So, current focus is getting on the components of indigenous medicine

to explore their bioactive potential [2]. *Lannea coromandelica* (Houtt.) Merr (Family: Anacardiaceae, subfamily: Spondiadoideae), synonym- *Lannea grandis* (Dennst.) Engl., *Odina wodier* Roxb., is a deciduous tree with thick, whitish to grey bark. Leaves crowded at the end of branches. The bark feels smooth and flakes off in small pieces when dry. The leaves are gathered from the wild for local use as food [3]. January to July is the flowering and fruiting time. The plant is also cultivated in some areas of the tropics as a hedge plant and roadside tree. The plant is distributed throughout the country and locally known as Bhadi, Jeol, Jial bhandi, Jhingangummi, Jhingam, Indian Ash Tree, Mandhol, Modhad, Miniyo, Moyno. It is also found in tropical moist and dry deciduous forests of Himalaya (Swat to Bhutan), Assam, Burma, Indo-China, Ceylon, Andamans, China, Thailand, Cambodia, Laos, Vietnam and Malaysia[4]. The tree is usually of small dimension in Bangladesh but is said to grow larger in more favorable climate. The leaf of the plant is reported to be used in the traditional medicine for the treatment of lipoma (Tumour), scurvy, tumour, ulcers, cancer, sprains, bruises, skin diseases, blood dysentery and dysentery[5,6]. The tree exudes a gum and the barks are also employed in native medicine [6]. Young leaves and sprouts - raw or cooked are eaten as a vegetable [5]. They are also eaten in lalab (a vegetable salad served with sambal) with rice [7]. In Mayanmer and some parts of the Madras Province in India, the leaves are used for all local swelling and pain [8]. The gum obtained from the trunk is often used in confectionery. The powdered bark is used as a flavouring agent. The bark contains tannins. It is used for the impregnation of fishnets [5]. Decoction of the bark is applied in toothache while the powdered form of the bark is used as toothpowder. The whole plant is used against elephantiasis, ulcers, coma, swelling, toothache and wounds. The leaves are used as astringent (Trivedi CP). The plant is used extensively in traditional herbal medicine of Bangladesh to treat various ailments including pain, inflammation and some other infectious diseases [10]. Jingini, Jhingan and Gudamanjari are Ayurvedic preparations that contain extract of this plant [11]. The leaves of *L. coromandelica* are copped as fodder and fed the livestock in inflammatory and digestive problems [12,13]. The bark is also considered astringent and stomachic; used as a lotion in impetigenous eruptions, leprous and obstinate ulcers; 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 [9, 10].

In a previous studies, the stem bark of *L. coromandelica* showed zoosporocidal activity (MIC 0.1 µg/ml) and its polyflavonoid tannins were credited for this activity [14]. A phytochemical report indicated the presence of β-sitosterol, physcion, anthranol B and dihydroflavonols in stem bark, flavonols and ellagic acid in leaves, leucocyanidin together with some leucodelphinidin in heartwood [15,16]. The ethanol extract of bark was reported to have antibacterial capacity against multi-drug resistant bacteria [17]. In pharmacological studies, stem bark indicated antioxidant and analgesic activity [18] while bark extracts indicated wound healing and antimicrobial activity[19]. The traditionally used plant extracts are experimented throughout the world on different biological and chemical models by the researchers to explore their phytoconstituents and therapeutic potentials. The traditional uses and the aforementioned biochemical evidences guided us to investigate the leaves and stem bark of *L. coromandelica* with advanced procedures. As a part our ongoing research[20-26], the present study is designed to provide scientific evidence for its use as a traditional folk remedy by investigating the phytochemical and analgesic activities against standard chromatogenic reagents and swiss-albino mice- *Mus musculus* respectively for the first time both for leaves and stem bark.

Materials and methods

Plant materials

Fresh leaves and stem barks were collected from Bania Khamar, near Nirala, Khulna, Bangladesh in July, 2014 with the help of a local traditional practitioner. The plant parts were collected carefully, keeping all of their macroscopic and microscopic identifications intact. Only those parts that have no visual discoloration, fungal or insect infestation were collected. The exsiccated plant samples were mounted on herbarium sheet and deposited and the species was confirmed by Sarder Nasir Uddin, Principle Scientific officer, Bangladesh National Herbarium, Mirpur, Dhaka. Herbal authentication was carried out by matching the arrangement of leaves with the stem, flower and fruits in the herbarium sheets of the plant with the preserved samples and pictorial glossary at the BNH repository. The voucher specimen of the plant has been deposited and preserved in BNH library for

further collection and reference and an accession no was provided as DACB-31,194.

Extraction

Extraction was carried out according to the method of Ahmed el al. 2008 [20] and Rahman et al 2013[21]. The collected plant parts were separated from undesired materials. They were shade dried with open air access for two week. The dried plant parts were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powders of the plant parts were stored in an airtight container and kept in a cool, dark and dry place until the analysis commenced. While conceding the study, 800 g of each powered materials were taken in some clean, dried, flat-bottomed glass containers and soaked in 99.8% ethanol. The containers along with their contents were sealed and kept for a period of 10 days with occasional shaking or stirring in a dark place. The mixtures then underwent a coarse filtration by cotton and whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The extraction procedure was repeated three times. The filtrates were combined and the plant residue was brought to dryness at normal room temperature using an electric fan facilitating evaporation of the solvent. The yield value was calculated by using the equation: % yield = (We/Wp) × 100; where, We = weight of dried extract and Wp = weight of dried powder. The yields of the extracts were found to be 22.2%, and 27.0% w/w for leaves and stem bark respectively of the dry powder weight which were designated as crude ethanol extracts. All the extracts were preserved in a refrigerator till further use.

Chemicals

Standard chromogenic reagents Lead acetate, Potassium dichromate, Ferric chloride, Hydrochloric acid, Sulphuric acid, Mayer's reagent, Dragendroff's reagent, Wagner's reagent, Hager's reagent, Molisch reagent, Benedict's reagent and Fehling's solutions used for preliminary phytochemical chemical group test were of reagent grade and purchased from Sigma-Aldrich Co. LLC, Missouri, United States. Dichlofenac sodium, used as a standard drug in the analgesic assay was collected from the Techno Drugs Limited, Bangladesh. Ethanol (≥99.8%; Reagent grade, Merck KGaA, Darmstadt, Germany) was used as solvent in maceration of the plant materials. Dimethyl sulfoxide (DMSO, ≥99.9%, BioReagent, for molecular biology; Sigma-Aldrich, India) was used to dissolve the extracts. Acetic acid, the pain inducer in experimental animal model was purchased from

Merck (Darmstadt, Germany).

Instruments and equipment

Electronic balance (serial no.- 1508, OHAUS, Germany) was used for this study. Glass made hatching tank, air pump and cover lamp to grow shrimp were purchased locally. Pipettes, Micro-pipette, test tubes and other glass apparatus used were of laboratory standard and procured from authorized dealer.

Test animals and their acclimatization

Young Swiss-albino mice- *Mus musculus* of either sex of 3–4 weeks of age, weighing 20-25 g were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for in vivo analgesic screening. The animals were housed in plastic cages in standard environmental conditions at the animal house in Pharmacy Discipline, Khulna University, Bangladesh for adaptation after their purchase under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2.0 °C and 12 h light-dark cycle). They were fed with standard vital feed (ICDDR, B formulated grower pelletized) and water was available ad libitum throughout the period of acclimatization. The research was carried out according to the rules governing the use of laboratory animals as acceptable internationally, comply National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and Organization for Economic Co-operation and Development (OECD) guidelines and the experimental protocol was approved by the Animal Ethics Committee, Khulna University (Ref: KU/Pharm/AEC-2005).

Preparation of sample

The suspension of the each test sample was prepared at the dose 250 mg/kg body weight. The extract was triturated in unidirectional manner by the addition of small amount of tween-80. After proper mixing of extract and tween 80 the distilled water was slowly added. The final volume of the suspension was made 5.0 ml. For the preparation of diclofenac at the dose of 25 mg/kg-body weight, 12.5 mg of diclofenac was taken and a suspension of 5.0 ml was made.

Phytochemical group tests

The dried leaves and stem bark extracts were dissolved in 99.8% ethanol and phytochemical groups present in the solutions were screened by

using standard test procedures outlined by khatun et al. 2013[22] and Rahman et al. 2015[23]. The preliminary phytochemical screening of the crude ethanol extracts was carried out by using standard chromogenic reagents- lead acetate, potassium dichromate, ferric chloride, hydrochloric acid, sulphuric acid, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Hager's reagent, Molisch reagent, Benedict's reagent and Fehling's solutions were used to detect steroids, alkaloids, gums, flavonoids, saponins, tannins, and reducing sugars using standard protocol. The colour intensity or the precipitate formation was used as analytical responses to these qualitative tests. 10% (w/v) solution of the extract in ethanol was used for each of the above test.

Determination of analgesic activity by acetic acid induced writhing method

The method of Rahman et al. 2015 [23] was adopted with minor modification for the analgesic activity study for the crude extract using the acetic acid-induced writhing model in mice. The animals were divided into control, positive control and test groups with six mice in each group. The animals of test groups received the plant extract at the dose of 250 mg/ kg body weight each of each extract. The positive control group was treated with diclofenac Na (standard drug) at a dose of 25 mg/kg body weight and the vehicle control group was treated with 1% tween 80 in distilled water at a dose of 10 ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 30 minute before intraperitoneal administration of 0.7 % (v/v) acetic acid solution (0.1 ml/10 g body weight) to induce abdominal contractions or writhing. Five minutes after the administration of acetic acid, the number of writhing (constriction of abdomen, turning of trunk and extension of hind legs) for each animal was counted for 15 minute. The number of writhing in the control was taken as 100% and percent inhibition was calculated as follows:

$$\% \text{ Inhibition of writhing} = 100 - \left(\frac{\text{treated mean}}{\text{control mean}} \right) \times 100$$

Results

Phytochemical tests

In the present study, the sample solutions were tested to determine whether steroidal compounds, tannins, gums and reducing sugars, alkaloids, flavonoids, gums and saponins were present or not; because, certain phytochemical groups are responsible for specific pharmacological actions. The

leaves extract showed the negative interference in carbohydrate test as well as in the saponins whereas only saponins was absent in the stem bark extract. Besides, both the extracts were found to contain steroidal compounds, tannins, gums, reducing sugars, alkaloids as well as flavonoids. Saponins were found to be absent in both samples (Table 1).

Analgesic activity by acetic acid induced writhing method

At the dose of 250 mg/kg of body weight, the leaves and stem bark extracts produced 7.08 and 4.86 % of writhing respectively in test animals in both dose dependant and time dependant manner in the acetic acid-induced writhing test (Table 2). The results were statistically significant ($p < 0.005$) and was comparable to the standard drug (diclofenac Na) which showed 21.46 % of writhing at a dose of 25 mg/kg body weight (Figure 1). It means, at the dose of 250 mg/kg of body weight, the leaves and stem bark extracts produced 95.14 and 92.92 % of writhing inhibition respectively whereas the standard drug (diclofenac Na) showed 78.54 % of writhing inhibition at the aforementioned doses (Figure 1). It implies, both the extracts produced better analgesia in the test animals than the standard drug.

Discussion

The fight of mankind against pain started from the beginning of the history. Still now, painkiller drugs are one of the highest consumed class of drugs. So, the quest of analgesic drugs both from natural and synthetic sources is the demand of time. In the present study, both the extracts showed the presence of alkaloids, tannins, gums, saponins and flavonoids. The leaves of the plant were reported to contain flavonols and ellagic acid, leucocyanidin, leucodelphinidin [15, 16]. Therefore, the plant is rich in secondary metabolites that often are responsible for observed bioactivities of the plant extract.

Plants that belong to family – Anacardeaceae is a rich sources of various biologically active substances with strong pharmacological activity. Besides, some other species of *Lannea* were reported for possessing strong medicinal possessions: *Lannea alata* was reported to have antibacterial and antioxidant activities of flavonoids; [27]; however, the identity of highly active constituents and their mechanisms of action are not clear. These species contains very important compounds like alkaloids, flavonoids, tannins and so on. The secondary plant metabolites steroids, alkaloids, flavonoids, tannins

were reported to have cytotoxicity in different cell lines [28-32]. In the present report, both of the plant parts showed to possess important bioactive compounds namely steroidal compounds, tannins, gums, reducing sugars, alkaloids as well as flavonoids along with good analgesic activity. However, further studies are required for isolation and identification of bioactive constituents and to observe their effects. Moreover, in many instances flavonoids, steroids, alkaloids and tannins isolated from medicinal plant extracts were reported to have antibacterial activity [24-26, 30]. Crude extract is the complex mixture of pharmaceutically important phytoconstituents along with many inactive substances. Both the 250 mg/kg crude leaves and stem bark extracts showed significant analgesic potentials activities against test animals compared to the standard of the dose 25 mg/kg body weight. The constituents present in the extract may responsible to produce analgesic activity. The pure compound may produce more potent activity if could be isolated. In the present study, scientific rational of the traditional uses of the plants is established for their analgesic properties. The extract caused both dose and time-dependent antinociception against chemical induced nociception (pain) in mice. Acetic acid causes inflammatory pain by inducing capillary permeability. The extracts showed significant effect in these pain inductions and suggests that their analgesic effect may in part be related to its anti-inflammatory neurogenic and narcotic properties [33]. This depicts that the studied plant can be a source of new or even noble analgesic as well as anti-inflammatory agents. However, further research should be continued for isolation and identification of individual compounds and to determine their specific activity.

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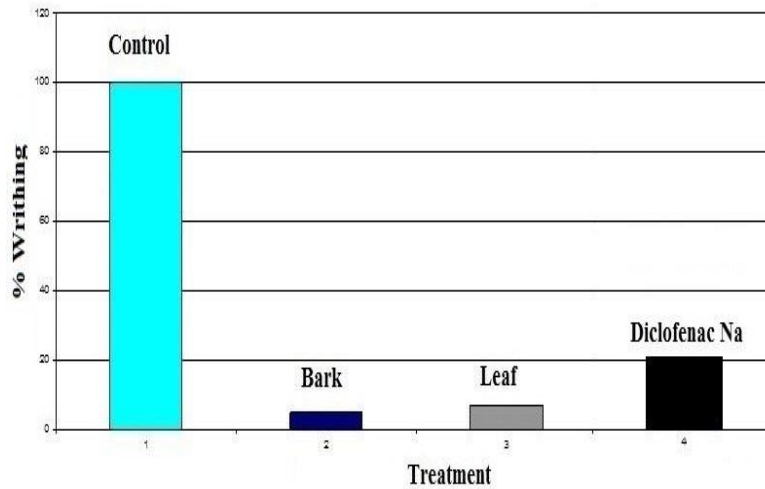


Figure 1. Effect of *Lannea coromandelica* (Houtt.) Merr. Linn. extracts on acetic acid induced writhing of mice. Each Bar represents % writhing produced by samples fed. Stem bark and leaf extracts were applied 250 mg/kg body weight whereas standard diclofenac Na 25 mg/kg body weight was applied on experimental animal model.

Table 1. Result of major phytochemical group test

Test for major phytochemical groups	Reagent/test	Ethanol extract of <i>Lannea coromandelica</i> leaves	Ethanol extract of <i>Lannea coromandelica</i> stem bark
Reducing sugar	Fehling's test	-	+
	Benedict's test	-	+
Alkaloid	Mayer's test	+	+
	Dragendorff's test	+	+
	Hager's test	+	+
	Wagner's test	+	+
Steroid and terpenoid	Salkowski's test	-	+
	Libermann-Burchard reagent	+	+
Tannin	Ferric chloride test	+	+
Gum	Molisch's test	+	+
Flavonoid	Shinoda test	+	+
	Alkaline reagent test	+	+
Saponin	Frothing test	-	-

“+” indicates presence and “-” indicates absence.

Table 2. Effects of *Lannea coromandelica* extracts on acetic acid induced writhing of mice.

Animal group	Treatment	Writhing (Mean±SEM)	% Writhing	% inhibition
Group I	1% tween solution in water	49.4± 0.43		
Group II	Diclofenac sodium (25 mg/kg bd. wt.)	10.6±08*	21.46	78.54
Group III	Leaves extract (250 mg/kg bd. wt.)	2.4±3.64*	4.86	95.14
Group IV	Stem bark extract (250 mg/kg bd. wt.)	3.50±0.76*	7.08	92.92

bd. wt.= body weight; SEM = standard error of mean; (n = 6); *P<0.005