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ORIGINAL ARTICLE

The Methanolic Crude Extract of *Garcinia lancifolia* Have Significant Pharmacological Activities With Potential Sources of Phytochemicals

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ABSTRACT

Background: *Garcinia lancifolia* (*G. lancifolia*), a potential medicinal plant has significant local use to alleviate various diseases like dysentery, diarrhea, dyspepsia, and as a pain reliever but not scientifically proven at yet. The present study was aimed to evaluate the anti-diarrheal, analgesic, and antioxidant activities of crude extract of *G. lancifolia*.

Methods: The plant parts of *G. lancifolia* were collected, dried, powdered, and extracted with methanol. Then the extracts were subjected to *in-vivo* anti-diarrheal activity by castor oil-induced method and analgesic activity by hot plate method in mice model. The Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and total phenolic content were demonstrated for the analysis of antioxidant activity.

Results: Phytochemical screening confirmed the presence of active phytochemicals like flavonoids, saponins, alkaloids, cardiac glycoside, and terpenoids in the plant extract. The extract at doses of 200 and 400 mg/kg showed significantly a reduction of diarrheal feces by 61.161% ($p < 0.01$) and 72.33% ($p < 0.001$), respectively in contrast to standard drug loperamide (77.83% reduction). In the hot plate method, the crude extract (300 mg/kg) revealed significant ($p < 0.05$) analgesia in comparison to standard aceclofenac. In an antioxidant activity test, the extract contains a moderate level of phenolic content 10.78 µg/mg of gallic acid equivalent and the antioxidant activity by using DPPH free radical scavenging showed poor antioxidant potential with an IC_{50} value of 148.26 µg/mL.

Conclusion: The findings of this study conclude that this plant is a potential source of pharmacological actions that may be a basis for further investigation on a large scale.

Keywords: Analgesics, Antidiarrheal, Antioxidants, Clusiaceae, *Garcinia lancifolia*.

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INTRODUCTION

Oxidative stress, an imbalance condition between antioxidants and free radicals and other reacting oxygen species, with an augmented level of free radicals may generate many degenerative diseases, like brain dysfunction, cardiovascular diseases, coronary arteriosclerosis, declination of the immune system, cancer, gastric ulcer, and aging processes.[1–4] To alleviate those free radical related disorders tocopherols, carotenoids, and ascorbic acid are the antioxidants have been used extensively. Recent study data reported that the best sources of natural antioxidants are always vegetables, fruits, seeds, cereals, wine, tea, onion, olive oil, berries, and aromatic plants, etc. Also, they have analgesics, anti-inflammatory and anti-cancer properties.[5]

Diarrhea, asymptomatic disease in the intestinal tract usually triggered by a variety of bacterial and viral infections. It is the second leading root of death in children younger than five (16%), afterward pneumonia (17%) in this age group.[6,7] The literature reviewed data prevalent that childhood diarrhea mostly in Africa and South-East Asia, with 696 million and 1.2 billion cases, respectively, whereas only 480 million in the rest of the world. From ancient times,

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plants are used by local communities and traditionally for managing diarrhea and other stomach disorders.[8,9] An report by World Health Organization (WHO), estimated worldwide 80% of individuals, are dependent on drugs from plant sources,[10] and around 30% of drugs were achieved primarily or secondarily from plants.[11] Among the urban and rural poor populations of Bangladesh, diarrhea is so much prevalent and prominent cause of death in children. Most similarly, with other developing countries, Bangladeshi people are habituated with traditional medicine for the managing of diarrhea. An ethnomedicinal survey revealed that above 250 floral species are used by folk and tribal medicinal therapists for the treatment of diarrhea.[12] Among them, *G. lancifolia* Roxb. is a prevalent medicinal plant that has plentiful ethnomedicinal uses by the different ethnic groups.

G. lancifolia Roxb. (Family: Clusiaceae), this medicinal plant is commonly known as Rupahi-thekera (Assamese), Pelh (Mizo), Rupohi tekera (Mising), widely distributed in the southern part of Bangladesh, Assam, and Meghalaya. To date, it is facing the danger of extinction in the environment and is frequently cultivated at homestead.[13,14] This small, attractive evergreen tree is long up to 12 feet under the dense shade of other trees. In Northeast India, the fruits and young leaves *G. lancifolia* are eaten as fresh vegetables or made into pickles.[15] Traditionally, this plant has been used to treat various disorders like dysentery, dyspepsia, fever, jaundice, diabetes, urinary problems, and as a stomachic, diuretic.[15] Moreover, previous data reported that the ripe fruits of *G. lancifolia* are indigenously used in worm infestations, to treat stomach disorders and diarrhea in Indian *Ayurvedic* system and the management of dysentery and diarrhea by the Karbi and Mishing tribes of Assam.[14] Despite the traditional use of this plant, pharmacologically this plant has validated in vitro antioxidant, anthelmintic, and antibacterial and in vivo analgesic, anti-inflammatory activity.[14–16]

Therefore, as a basis of extensive folkloric uses, this study was planned to validate the anti-diarrheal, analgesic activity in mice model, and the antioxidant activity of methanolic extract of *G. lancifolia* whole plant for the first time in Bangladesh.

MATERIALS AND METHODS

Drugs

Aceclofenac (ACI Pharmaceuticals Ltd., Bangladesh), castor oil, loperamide (Square Pharmaceuticals Ltd., Bangladesh), and normal saline (Opsonin Pharmaceuticals Ltd., Bangladesh) were purchased from the declared suppliers.

Experimental Animals

For performing in vivo pharmacological experiments, Swiss-albino mice, a weight of 20-25 g on average either sex (aged 4-5 weeks) were procured from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). Animals were provided controlled temperature in the room (24 ± 2º C; RH 60-70%) for twelve hours and fed ICDDR; B prepared food, and water Animals were kept before the test for at least 3-4 days in the environment. Fasting was performed for eighteen hours before performing an experiment. The Institutional Ethical Committee of Noakhali Science and Technology University (Ref-2015/BKH1203MS121) approved the planned protocol of this study. Handling and care of the experimental animals were according to the international guidelines of the National Research Council.[17]

Plant Materials

The whole plant of *G. lancifolia* Roxb. was collected from Moheshkhali, Bangladesh in April 2014. It was recognized and authenticated by Naimur Rahman, a scientific officer in the Bangladesh National Herbarium (DACB), Mirpur-1, Dhaka with certification number is 38329, where the plant was dumped for future identification.

Plant Extraction

About 238 g of crushed powder of the whole plant was soaked in 900 ml of 80% methanol (Merck, Germany) in a fresh, flat-bottomed glass bottle. This well-closed bottle was taken for 15 days accompanying irregular shaking and stirring. Firstly, the content of the mixture was filtered using clean and white cotton fabric and, finally, by a Whatman No. 42 filter paper. The final filtrates were kept at room temperature in a laboratory for evaporation. A sticky concentrate of light greenish color plant extracts was found and preserved at 4°C until analysis.

Phytochemical Screening

The newly prepared crude methanolic extract was examined for the existence of alkaloids, glycosides, gum, flavonoids, phenols, diterpenes, proteins, tannins, saponins, phytosterol, etc. The availability of these mentioned chemical components was recognized by distinguishing change in color using the standard method of phytochemical screening procedures.[18,19]

Anti-diarrheal Activity

The anti-diarrheal activity was performed in this study described by Shoba and Thomas[20] with slight modification.

At first, mice were grouped into control, positive control, and test groups containing five mice in each group. Control group treated with vehicle (1% Tween-80 in normal saline) at a dose of 10 mL/kg orally and positive group with loperamide at the dose of 5 mg/kg orally. The testing group was received plant extract at a dose of 200 mg/kg and 400 mg/kg body weight. Each animal was placed in an individual cage; the floor lining was changed at every hour. Diarrhea was induced by oral administration of castor oil to each mouse after the above treatment. During an observation period of 5 hours, the number of diarrhoeic feces excreted by the animals was recorded.

Analgesic Activity

The central analgesic activity of the plant extract was performed by the hot plate method. In this method, the test animals were divided into five groups of five mice each. Three different groups of mice received orally 100, 200, and 300 mg/kg of body weight of the extract. Aceclofenac (20 mg/kg) was administered orally to the positive control, and distilled water (10 mL/kg) was given to the control group. One hour after treatment, the animals were placed on a hot plate maintained at 55 ± 2°C. The time taken by the mice to start licking the paw or jump out of the hot plate was considered as the reaction time.[21] Assessment of analgesic responses was determined at 60, 90, 120, 150, and 180 minutes after administration of the samples.

Antioxidant activity

Total Phenolic Content Determination

Folin-Ciocalteu, a stable reagent was used to access total phenolic content in the plant extract as a gallic acid equivalent according to the previously reported method.[18]

Free Radical Scavenging Activity by DPPH Method

A stable radical DPPH is an established method by Brand Willians et al. as previously described,[22] was used in this study to measure the free radical scavenging activity of *G. lancifolia* plant extract.

Statistical Analysis

The statistical analysis was done in this current study by using the SPSS software package (version 19.0). All computed values are expressed as mean ± SEM. Data analysis among the different groups was compared using one-way ANOVA followed by Dunnett's post hoc test. Asterisks indicate increasing levels of significance: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

RESULTS

Phytochemical Screening

A report on the phytochemical screening of *G. lancifolia* supported the existence of alkaloid, saponins, flavonoids, cardiac glycoside, terpenoids, phytosterol, and tannins (Table 1). Additionally, it is mentioned that the existence of alkaloids, saponins, and flavonoids extensively.

Table 1: Phytochemical screening of the methanolic extract of *G. lancifolia*

Name of phytochemical	*Observation*
Alkaloid	+ +
Cardiac Glycoside	+
Carbohydrate	-
Saponines	+ +
Triterpene	+
Phytosterol	+
Flavonoids	+ +
Protein and amino acids	-
Tannins	+

Here, (++) = presence of constituents extensively; (+) = presence of constituents; (-) = absence of constituents

Anti-Diarrheal Activity

The obtained results from the present study of the effect of methanolic crude extract of *G. lancifolia* on castor oil-induced diarrhea are presented in Table 2. The extract at a dose of 200 and 400 mg/kg significantly ($p < 0.001$) showed a reduction of diarrhea 61.16 and 72.33%, respectively, in test animals as compared to standard loperamide (77.83% reduction).

Table 2: Anti-diarrheal activity of *G. lancifolia* by castor oil induced diarrhea in mice model

Treatment	*Dose (b.w.)*	*No. of diarrheal faeces (mean ± SEM)*	*% Reduction of diarrhea*	*P value*
CTL	10 ml/kg	6.00 ± 0.58	---	
STD	50 mg/kg	1.33 ± 0.33	77.83^{***}	0.000
ME 2	200 mg/kg	2.00 ± 0.58	61.16^{**}	0.001
ME 1	400 mg/kg	1.66 ± 0.33	72.33^{***}	0.000

Values are expressed as Mean ± SEM (n = 5). ***P < 0.001, **P < 0.01 compared to control (One way ANOVA followed by Dunnett's 't'-test); CTL: Control; STD: Standard; ME: Methanolic extract.

Analgesic Activity

The central analgesic activity possessed the methanolic extract of *G. lancifolia* using a hot plate test in Swiss albino mice was illustrated in Figure 1. The extract at a dose of 200 and 300 mg/kg body weight exhibited significant analgesic effect ($p < 0.05$) at 90 and 120 minutes after administration as compared with a control group, respectively, while aceclofenac showed significant analgesic activity at different time interval in contrast to control group.

Antioxidant Activity

The total phenolic contents of the plant extracts are presented in Table 3. Total phenolic compounds were stated as gallic acid equivalents by reference to a standard curve equation y = 0.0161x - 0.0065 with a regression coefficient value of 0.964. The results showed that the total phenol content of the extract was found to be 10.78 ± 0.43 mg/g, and this recommends that the plant may have moderate antioxidant activity. The existence of moderate free radical scavenging activity compares to the reference standard, also confirmed by the DPPH method (Figure 2).

DISCUSSION

Phytochemical screening report confirmed the presence of alkaloid, saponins, flavonoids, cardiac glycoside, terpenoids, phytosterol, and tannins as a natural product in the methanolic extract of *G. lancifolia* in accordance with other reported works.[15,16] The previous report stated the presence of phytochemicals in the plant extract, having a vigorous role in the management of various ailments.[23]

Ricinoleic acid is an active metabolite of castor oil, which is responsible for peristaltic activity in the

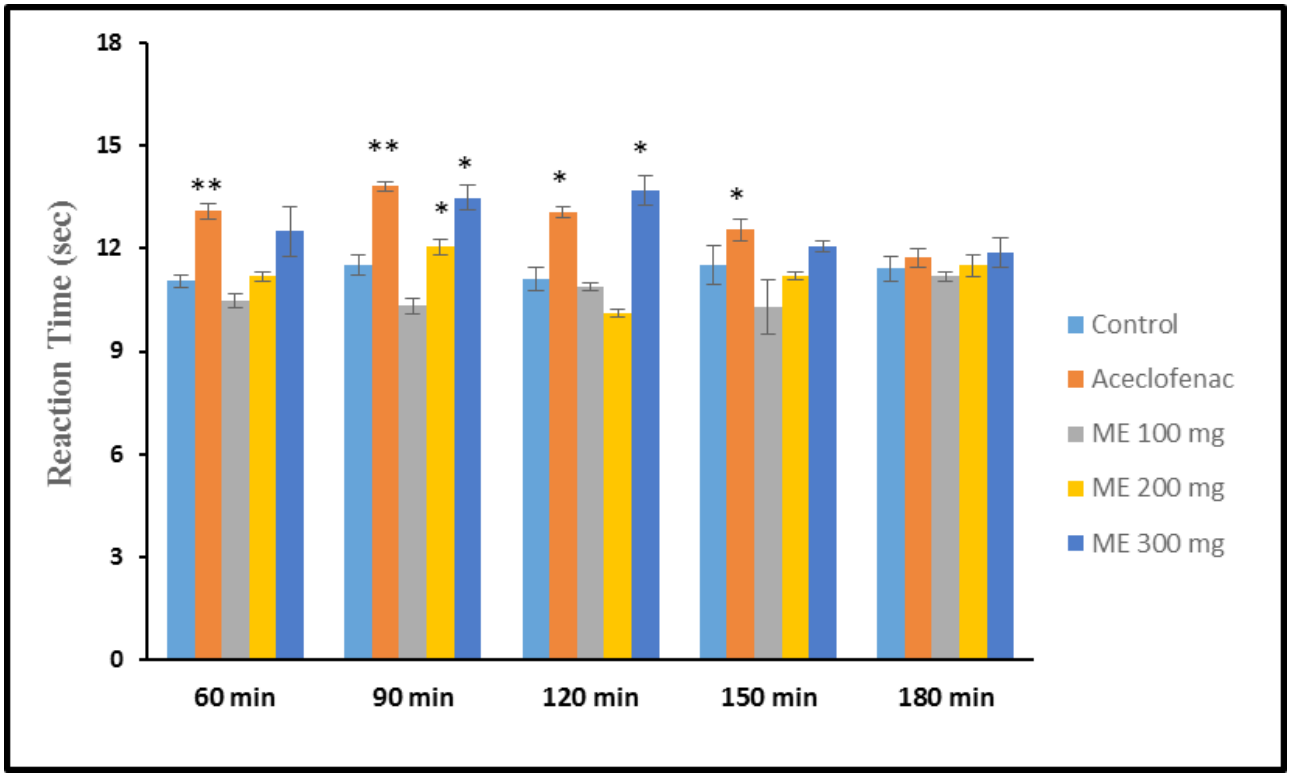

Figure 1: Analgesic activity of the methanolic extract of *G. lancifolia* by using hot plate method. Here, distilled water and aceclofenac were used as control and standard respectively; ME represent methanolic extract; each value represents the mean ± SEM (n=5). **P<0.01, *P<0.05 compared with control. (One way ANOVA followed by Dunnett's 't'-test).

Table 3: Amount of total phenolic content in *G. lancifolia* plant extract

Extract	*Absorbance of the sample*	*Average absorbance*	*Total phenolic content (mg/g) of GAE*
Methanolic extract	0.167		
Methanolic extract	0.170	0.168 ± 0.001	10.78 ± 0.43
Methanolic extract	0.168		

Results are expressed as mean ± SD (n = 3) of duplicate analysis

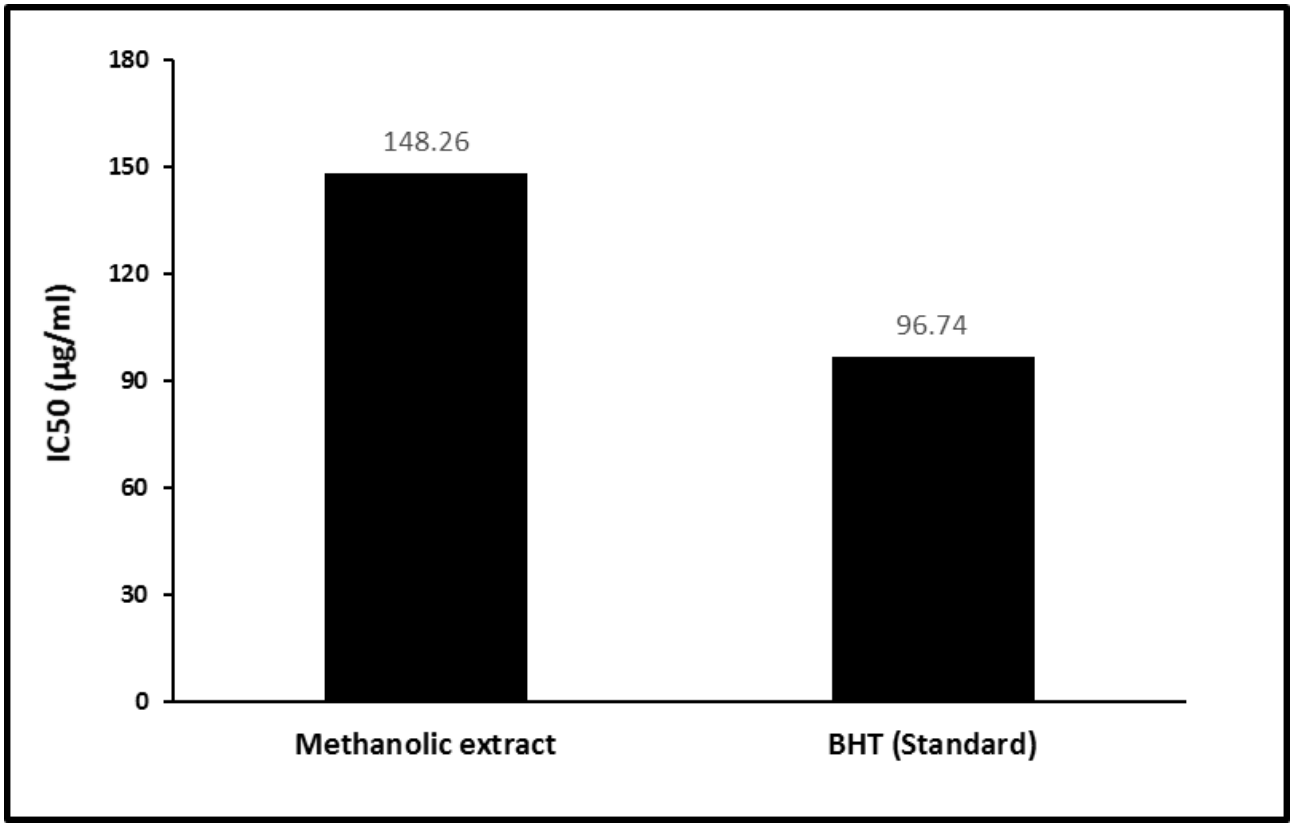

Figure 2: IC_{50} values of the standard BHT and sample methanolic extract of *G. lancifolia*

small intestine and alter the electrolyte permeability of the intestinal mucosa.[24] This metabolite of castor oil also produces an irritating and inflammatory action on the intestinal mucosa linked with stimulating the release of endogenous prostaglandin. In the present study, the extract exhibited significant anti-diarrheal effect in castor oil-induced mice model following other reported works like aqueous leaves extract of *Momordica charantia*[25] and methanolic extract of *Lantana camara.*[26] The former studies data confirmed that the presence of tannin,[27] flavonoids,[28] alkaloids,[20] saponins, and terpens[27] in the plant extract exhibited anti-diarrheal activity. Identified tannins in the plant extract are responsible for making the intestinal mucosa more resistant the diminish the peristaltic movements and intestinal secretions by creating protein tannates in the intestinal mucosa.[29] Therefore, the presence of saponins, alkaloids, and flavonoids in the crude extract of *G. lancifolia* are accountable for anti-diarrheal activity.

To evaluate the central analgesic activity by the hot plate method was employed in this study. This complex process of an established method is considered to be selective to observe compounds through opioid receptors.[30] The 'μ' is a proven potential opioid receptor in regulating thermal pain. Furthermore, stimulation of 'μ2' opioid subtype receptor leads to spinal analgesia.[31] It might be considered that the analgesic activity of *G. lancifolia* plant extract is likely to be mediated centrally while the exact mechanism is yet to be exposed. Ethnopharmacology studies of different plant extracts showed analgesic effects in mice models with the presence of phytochemicals like alkaloids, glycosides, flavonoids, and saponins.[32,33] In the present study, the titled plant extract exhibited significant analgesic activity due to the presence of the above-mentioned phytochemicals extensively.

The antioxidant activity of *G. lancifolia* was evaluated by using two most common methods; DPPH free radical scavenging and total phenolic content methods. The presence of reactive oxygen species, particularly free radicals in the human body, can initiate lipid peroxidation, which will lead to altered structure and function of collagen basement membranes that plays a role in diabetes mellitus, atherosclerosis, cell damage, cancer, myocardial infarction, etc.[34] The plant extract exhibited significant free radical scavenging properties and the total phenolic content

expressed as gallic acid equivalent. It was reported that the presence of phytochemicals like phenols in the plant extract is the probable reason for demonstrating the antioxidant activity.[35]

CONCLUSION

Based on the outcomes of our entitled study, it can be decided that the crude extract of *G. lancifolia* displays significant anti-diarrheal and analgesic activities with the active sources of potentially bioactive compounds. As this plant is used in traditional medicine, the extracts should be further explored for its phytochemical profile to identify active constituent responsible for that activity scientifically.

DECLARATIONS

Author Contribution Statement

Participated in research design: Sujan Banik, Jamiuddin Ahmed.
Conducted Experiments: Antara Ghosh.
Performed data analysis: Sujan Banik.
Wrote or contributed to the writing of the manuscript: Antara Ghosh, Sujan Banik.

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