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ASSESSMENT OF PRELIMINARY PHYTOCHEMICAL SCREENING, CYTOTOXIC AND HYPOGLYCEMIC ACTIVITY OF *LANNEA COROMANDELICA* BARK EXTRACT

Mohammad Iqbal Hussain [1], Somnath Shill [1], Subrato Kumar Barman [2], Khurshid Jahan [3]*

[1] Department of Pharmacy, [Jagannath University], Dhaka 1100, Bangladesh
[2] Department of Pharmacy,[Ranada Prasad Shaha University], Narayanganj 1400, Bangladesh
[3] Department of Pharmacy, [World University of Bangladesh], Dhaka 1205, Bangladesh
*jahankhurshid85@gmail.com

Abstract

The study was designed to evaluate cytotoxic, hypoglycemic potential and screening of phytoconstituents of the ethanolic bark extract (EBE) of *L. coromandelica* and its n-hexane soluble fraction (nHSF) and Ethyl acetate soluble fraction (EASF). Well-elucidated method was employed for our investigation. Moreover, The extractives weresubjected in cytotoxic and hypoglycemic activities. The cytotoxic activity was evaluated by using the nauplii of brine shrimp. In addition swiss albino laboratory mice were used for the study of hypoglycemic activities. Our estimated result confirms the presence of phytoconstituents like Flavonoids, Saponins, Carbohydrates, Triterpenes etc. However, Brine shrimp lethality bioassay showed significant cytotoxic effects with LC_{50} value 3.48±0.40 µg/ml, 2.78±0.049 µg/ml and 2.63±0.118 µg/ml, for EBE, EASF and nHSF compared to the standard Vincristine Sulfate (VS) with LC_{50} value 0.47±0.0005 µg/ml respectively after 24 hours. In the study of hypoglycemic activities, at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w., the EASF reduced blood glucose level by 31.53%, 33.43%, and 36.12% and nHSF reduced 15.03%, 17.65%, and 23.46% respectively at 120 minutes.There is a statistically significant difference in blood glucose level reduction between standard and test groups except nHSF 200 mg/kg b.w. Mean values of results were considered statistically significant when ($P < 0.05$). The estimated result indicated that EBE of *L. coromandelica* possess potential cytotoxic, and hypoglycemic activities.This study specified that the EBE of *L. coromandelica* could be an alternative of hypoglycemic and cytotoxic agents as well as source of potent bioactive compounds.

Keywords: *Lannea coromandelica, Hypoglycemic agent, Cytotoxic activities, Mice, Medicinal plants*

Introduction

Medicinal plants are considered as valuable source of therapeutic agents used in the treatment of multiple diseases throughout the world. They are also used in development and synthesis of new drugs, and development of human cultures. [1] The World Health Organization (WHO) always recommended and motivated the utilization of medicinal plants. [2] Moreover the medicinal values of most of the plants have not been investigated yet when they are considered as optimistic source of therapeutic agents. [3] The chronic metabolic disease diabetes mellitus is caused by improper production and functioning of insulin. [4] The disorder is associated with prolong damage and dysfunction of different organs, like eyes, heart, blood vessels, kidneys etc. [5] Medicinal herbs possess anti cancer agent for long period of time where the cytotoxic drugs usually kill cancer cells or modify their abnormal growth. [6] *L. coromandelica* family Anacardiaceae is a species of tree usually grows in Bangladesh, India and some other countries of Southeast Asia. [7] The plant is good as a traditional herbal medicine in Bangladesh to treat various conditions like inflammation, pain, and some infectious diseases. [8] The bark is deliberated as stomachic, astringent also used as a lotion in impetiginous eruptions, obstinate ulcer and leprous, skin eruptions, heart disease dysentery, and mouth sores. [9] Literature review noticed that with Bangladeshi origin very limited study has been done. Our current study features to evaluate the antidiabetic and cytotoxic activity of ethanolic extract of *L. coromandelica* bark.

Methods

Plant material collection

The bark of *L. coromandelica* plant was collected from Savar, Dhaka, in the year of December, 2019. The plant was identified by Bangladesh National Herbarium where a voucher specimen has been deposited with an accession number. The accession number is DACB 46307.

Extract preparation

The barks of *L. coromandelica* were cut into small pieces then air dried for several days under shade and was allowed to ground until sound observed during breakage. The dried barks were then ground to coarse powder.The powdered 400 gm bark was taken in a cleaned, amber colored glass reagent bottle and 5.5 liters of 99.95% ethanol was added to the bottle to soak the bark powder. The content in the bottle was kept for a period of about two weeks with occasional shaking and stirring. The whole mixture then filtered through a fresh cotton plug. The filtrate then filtered again with Whatman filter paper and total filtrate was concentrated also using a rotary evaporator to get the crude extract. The residue was then stored & used for our current investigations.

Solvent-Solvent Partition of Crude Extract

Solvent-solvent partitioning crude extract was done using the protocol designed by Kupchan and modified by Van Wagenen et al, (1993). [10] The 5.0 g crude extract was dissolved in 90 ml ethanol and 10 ml water (10% aqueous ethanol). Then it was partitioned and the resultant partitionates were evaporated to dry to yield nHSF and EASF. All fractionate samples were stored for further analysis.

Chemicals and Drugs

Brine shrimp eggs, Dimethylsulfoxide (DMSO), ethanol 99.95%, n-hexane, ethyl acetate, molisch reagent, benedict's reagent, sodium hydroxide solution, acetic anhydride, sulfuric acid, glibenclamide, glucose, tween 80 and other organic solvents used for our experiments were brought from local market, Dhaka, Bangladesh. All the chemicals and organic solvents were of analytical grade.

Phytochemical group tests

Using standard test procedures, phytochemical tests were accomplished for the identification of different phytoconstituents.

Test for carbohydrates [11]

For the test of carbohydrate, small amount of crude extract was dissolved in small amount of distilled water and then filtered. The filtrate was used for the detection of carbohydrates.

a) Molisch's Test:Some amount of filtrate was mixed with Molish reagent (alcoholic α-naphthol solution). The development of purple ring at the

junction by the concentrated acid is the confirmation of carbohydrates.

b) Benedict's Test:Some amount of filtrate was mixed with Benedict's reagent (complex mixture of sodium carbonate, sodium citrate and copper ii sulfate pentahydrate) and then heated gently. Formation of Orange precipitation confirms the presence of reducing sugars.

Test for saponins [12]

Foam Test: Some amount of crude extract when mixed with small quantity of water and shaken strenuously, formation of layer of foam is the confirmation result for saponin.

Test for flavonoids [13]

Alkaline Reagent Test: Small quantity ofalcoholic crude extract was mixed with one or two drops of sodium hydroxide solution. Deep yellow colour was formed but becomes colourless on addition of dilute acid confirms the presence of flavonoids.

Test for tannins

Ferric chloride test:Alcoholic crude extract when mixed with 2-3 drops of ferric chloride solution, Blackish blue color precipitation formation specified positive result for tannins.

Test for steroids

Liberman- Burchard test:The mixture of crude extract and chloroform was filtered and with the filtrate, some drops of acetic anhydride was mixed well. After that 1.0 ml of concentrated sulphuric acid was added very carefully. Green color formation is the indicator of steroids.

Detection of Triterpenes [14]

Salkowski test: Some amount of crude extract was mixed with small amount of chloroform and concentrated sulphuric acid. The mixture was done very carefully so that a layer is formed. At the junction, reddish brown colour formation is the confirmation of the presence of terpenoids.

Cytotoxic investigation

The Cytotoxic investigation of the extract and its fractionate sample was accomplished on brine shrimp nauplii using method of Mayer et al (1982). [15] 20.0 mg of extractive sample was dissolved in 1.0 ml DMSO. Then 38.0 g of sodium chloride (NaCl) was weighed out and dissolve in 1L distilled water to prepare the saline solution. After collection of brine shrimp eggs, it was hatched to mature as nauplii. 8.0 mg of *L. coromandelica* bark extract and its fractionate samples were dissolved in 100 µl DMSO and then diluted to several concentrations (200 µg/ml, 100 µg/ml, 50.0 µg/ml, 25.0 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml and 0.78125 µg/ml) respectively. The measured amount of VS was dissolved in DMSO to get an initial concentration of 20.0 µg/ml from which serial dilutions were made to get 10.0 µg/ml, 5.0 µg/ml, 2.5 µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml, and 0.0390 µg/ml respectively. VS was used as the positive control. Observation was done after 24 hours. The percentage of mortality was calculated for each sample and by plotting the values of percentage of mortality and log of sample concentration, the LC_{50} was measured.

Hypoglycemic activity

Animals

Swiss-albino male and female mice were obtained from International Centre for Diarrheal Disease and Research, Bangladesh (icddr,b). They were 6-7 weeks old, weighing 20-25 g on average. Standard laboratory feed and freshwater were supplied and the animal were acclimatized for 7 days at the laboratory, Department of Pharmacy, Jagannath University. The experimental animals then kept in ventilated room and temperature of (25 ± 2)°C was maintained at day-night cycles in quiet and soundless place. All procedures in connection with the experiment were performed in an ethicall way by following the European Community guiding principles in the care and use of animals. [16]

Experimental Procedure

The hypoglycemic activity of *L. coromandelica* bark extractwas accomplished in overnightfasted normal mice following the method described by Joy and Kuttan (1999). [17] The fasted mice were divided into eight groups of five in each. Different groups received drugs and test samples of different concentration like the control group received vehicle (3% Tween 80), the standard group received

Glibenclamide (10 mg/kg body weight), and groups 3-8 received the nHSF and EASF at different doses (100, 200, and 400 mg/kg) body weight respectively. Before oral administration of standard drug, and test samples, individual weight of each animal was recorded and dose was adjusted. Initial and after 60 & 120 minutes of glucose loading (2.0 g/kg b.w.), blood glucose level was measured by glucometer.

Statistical analysis

The results are expressed as mean ± S.E.M. (standard error of mean). One way-ANOVA analysis with post hoc Dunnett's test ($p < 0.05$, versus control) was performed for determining statistical significance. Pair-wise comparisons among the groups (without control) was also carried out with post hoc Tukey test ($p < 0.05$, versus standard/fractions). IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) was used for analyzing the data. [18]

Results

Chemical group screening

Phytochemical screening of *L. coromandelica* bark extract showed the presence of Flavonoids, Saponins, Carbohydrates and Triterpenes (Table 1).

Cytotoxicity study

The EBE and its fractionates of *L. coromandelica* showed cytotoxic activities comparing toVS (Table 2). the lowest LC_{50} values symbolized the more toxic effects. [19] LC_{50} values were evaluated from the straight line obtained by plotting the log of sample concentrations versus % mortality.

Hypoglycemic activity

The blood glucose level reduced by *L. coromandelica* at different times in different groups is shown in (Table 3). At doses of 100 mg, 200 mg, and 400 mg/kg b.w., the EASF reduced blood glucose level by 31.53%, 33.43%, and 36.12% and nHSF 15.03%, 17.65%, and 23.46 % respectively at 120 minutes which were not statistically significant except nHSF (200 mg/kg b.w.).Both nHSF and EASF reduced blood glucose level in a dose dependent manner at 60 and 120 minutes. There is a statistically significant difference in blood glucose level reduction between standard and test groups except nHSF 200 mg/kg b.w. EASF showed more comparable hypoglycemic activity than nHSF with standard. The observation indicated that the plant is potential for further scientific studies towards the discovery of novel antidiabetic drugs.

Discussion

Our current study confirms the presence of phytoconstituents like Flavonoids, Saponins, Carbohydrates, Triterpenes etc. However, the plant was reported to contain phytoconstituents like carbohydrates including Gums, Proteins, Terpenoids, Polyphenols, Flavonoids.[20] Triterpenoid saponins are the triterpenes that are important component for retarding proliferation of cancer cell and promoting apoptosis. [21] So presence of this component confirms the cytotoxic property of the *L. coromandelica* bark extract. In our current study, there was a dose dependent increase in cancer preventive activity.

Islam et al. (2018) showed antidiabetic activity of EBE of *L. coromandelica.* [22] Hence, this study was performed to evaluate the hypoglycemic activity of nHSF and EASF of ethanolic extract of *L. coromandelica* bark. Flavonoids, alkaloids, sterols/triterpenoids, and phenolics are considered to be bioactive antidiabetic principles. [23] Our observed hypoglycemic activity may be due to the presence of one or more of these bioactive components in the extract. Our conducting work confirms that the plant is a potent source of alternative medicine and can play vital role in health care system. Further studies on bioactivity-guided isolation of active principle and their exact mechanism of action is needed toward discovery and development of novel antidiabetic as well as anticancer drugs.

Conclusion

The nHSF and EASF of bark extracts of *L. coromandelica* confirmed the presence ofdifferent groups of phytochemicals. They showed significant cytoprotective effects also. The hypoglycemic activity was also reportedly significant. Both of the nHSF and EASF at higher concentration was seen to have the cytoprotective as well as hypoglycemic effects. In conclusion, this study hands over clear evidence for the ethnobotanical uses of *L. coromandelica* bark extract in the cure of diabetes mellitus. Our estimated result lead us more deep insight to isolate and recognise the responsible

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bioactive molecule and its respective *in-vivo* study in further research.

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Conflict of interest statement

There is no conflict of interest to declare.

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Table 1. Results of phytochemical screening of *L. coromandelica* bark extracts.

Chemical constituents	Test methods	Result
Carbohydrates	Molisch's test	+
	Benedict's test	+
Saponins	Foam test	+
Falvonoids	Alkaline Reagent test	+
Tannins	Ferric Chloride test	-
Triterpenes	Salkovaski test	+
Steroids	Liberman-Burchard test	-

+ Symbol indicates presence and – indicates absence with respect to extractive solvents

Table 2. LC50 values of cytotoxicity study of *L. coromandelica* bark extracts expressed as (Mean ±S.D, n=3)

Sample	LC_{50} (µg/ml)
EBE	3.48±0.408 µg/ml
EASF	2.78±0.049 µg/ml
nHSF	2.63±0.118 µg/ml
VS	0.47±0.0005 µg/ml

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Table 3. Effect of different doses of nHSF and EASF of *L. coromandelica* bark on blood glucose level (mmol/L) of mice.

Groups	Blood glucose level at minutes after the treatment		
	0 min	60 min	120 min
Control	5.62 ± 0.72	5.98 ± 0.67	6.14 ± 0.65
Standard	6.78 ± 0.73	3.86 ± 0.29*°■•∆▲ (43.07 %)	2.62 ±0.20*°■•∆▲ (61.36 %)
nHSF (100 mg/kg)	6.52 ± 0.30	6.26 ± 0.24θ (3.99 %)	5.54 ± 0.27θ (15.03 %)
nHSF (200 mg/kg)	4.76 ± 0.47▲	4.48 ± 0.29■•∆▲ (5.88 %)	3.92 ± 0.51* (17.65 %)
nHSF (400 mg/kg)	7.16 ± 0.78	6.92 ± 0.76θ□ (3.35 %)	5.48 ± 0.50θ (23.46 %)
EASF (100 mg/kg)	7.04 ± 0.58	6.58 ± 0.38θ□ (6.53 %)	4.82 ± 0.26θ (31.53 %)
EASF (200 mg/kg)	7.24 ± 0.42	6.46 ± 0.44θ□ (10.77 %)	4.82 ± 0.41θ (33.43 %)
EASF (400 mg/kg)	8.86 ± 0.94*□	6.84 ± 0.33θ□ (22.80 %)	5.66 ± 0.65θ (36.12 %)

Results are expressed as mean ± S.E.M. (n = 5); *$p< 0.05$ versus Control (Dunnett t-test); θ$p< 0.05$ versus Standard; °$p< 0.05$ versus n-hexane (100 mg/kg); □$p< 0.05$ versus n-hexane (200 mg/kg); ■$p< 0.05$ versus n-hexane (400 mg/kg); •$p< 0.05$ versus Ethyl acetate (100 mg/kg); ∆$p< 0.05$ versus Ethyl acetate (200 mg/kg); ▲$p< 0.05$ versus Ethyl acetate (400 mg/kg) (pair-wise comparison by post hoc Tukey test); the values in parentheses represent the percentage reduction in blood glucose level from 0 min in the respective group.