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Exploration of Phytochemical and Pharmacological Potentials of *Canarium resiniferum* Bruce ex King, an Endangered Medicinal Plant of Bangladesh

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ABSTRACT

Canarium resiniferum Bruce ex King is an endangered medicinal plant of Bangladesh that has a lot of beneficial uses in folk medicine. The study investigated the phytochemical composition and the level of thrombolytic, anti-inflammatory, antipyretic, analgesic and hypoglycemic activities of hexane, chloroform and aqueous soluble fractions of the methanol extract of *C. resiniferum* leaves. Phytochemical analysis was performed by standard methods. In the thrombolytic assay, the chloroform fraction (500 µg/100 µL) of *C. resiniferum* exhibited significant ($p<0.01$) thrombolytic activity by human blood clot lysis of 57.32% as compared to the standard streptokinase (80.1%). The chloroform soluble fraction also showed the maximum anti-inflammatory potential in both egg albumin denaturation assay and human RBC membrane stabilizing assay. Yeast-induced pyrexia was utilized to evaluate the antipyretic effect of different fractions of *C. resiniferum* at a dose of 500 mg/kg. The temperature reducing capacity of the test samples was prominent which persisted up to 3 h of administration of test samples in mice. In tail immersion method, the hexane fraction significantly ($p<0.05$) increased the pain threshold with 228.57% elongation at 90 min while the chloroform fraction showed maximum 29.82% inhibition of formalin-induced licking and biting responses in mice. In the oral glucose tolerance test, the extracts (500 mg/kg) also exhibited significant blood glucose lowering effect in mice compared to the control group. The findings in the study support the rationale uses of *C. resiniferum* as folk medicine in Bangladesh.

Keywords: Analgesic, Anti-inflammatory, Antipyretic, *Canarium resiniferum*, Hypoglycemic, Phytochemical, Thrombolytic.

Introduction

Medicinal plants are a source of bioactive leads that can produce substantial pharmacological action in biological system. Therefore, they can play a vital role in the discovery and development of new therapeutic lead compounds for the maintenance of human health.[1,2] Various plant extracts have been exploited for the study of anti-inflammatory, analgesic, antibiotics, anti-diabetic, cardioprotective and other active compounds that are beneficial to cure ailments and improve health. According to World Health Organization (WHO), 80% of the world's populations use folk medicine derived from plants for primary health care. About 25% of prescribed medicines today are derived from plants sources.[3] Bangladesh is an attractive storehouse of various medicinal plants among the South Asian countries. Most of these plants have been utilized for the preparation of Ayurvedic and Unani formulations in Bangladesh. However, the greater part of these plants has not yet undergone chemical and pharmacological studies to explore their bioactive lead(s). Consequently, the current study was designed for preliminary screening of phytochemicals and evaluation of thrombolytic, anti-inflammatory, antipyretic, analgesic and hypoglycemic activities of leaves of *Canarium resiniferum* Bruce ex King (Family-Burseraceae). *Canarium resiniferum* is a native rare tree species of Bangladesh. Locally, the plant is familiar as Dhup. The species is an aesthetically important and evergreen tree which naturally grows in the forests of Chattogram, Chattogram Hill Tracts, Cox's Bazar, Rangamati and Sylhet districts in Bangladesh.[4] In India, the species has been declared as an endangered plant by Indian institute of forest management. The plant has vast medicinal applications in different systems of traditional medicine.[5] The plant can produce a resin product, named as black Dammer resin, which is one of the major drugs in Siddha medicine. Traditionally the resin is used in the treatment of respiratory ailment and rheumatism.[6] Resin and its isolated compounds showed antimicrobial,[7] analgesic and anti-inflammatory activities.[8] The essential oil obtained from the black Dammer resin exhibited a potent anti-inflammatory activity in mice model.[6] In a recent work, antipyretic activity of the methanol leaf extract of *C. strictum* was established by yeast-induced pyrexia method in Wistar rats.[9] Although, there are several reports published on the biological functions of resin product of *C. resiniferum*, sufficient experimental data on many other pharmacological properties of its leaf extract are still not available. In continuation of our research studies on medicinal plants[10,11] the current work was carried out for the phytochemical and pharmacological investigations of *C. resiniferum.*

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Materials and Methods

Plant materials

Leaves of *C. resiniferum* were obtained in January 2019 from Bangladesh Forest Research Institute (BFRI), Chattogram, Bangladesh where its identification (Accession no: BFRIH-472/SA) was performed by a taxonomist Mr. Md. Syedul Alam.

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Extraction and partitioning process

The collected leaves were cleaned, dried in an oven at 40 ^{o}C and then subjected to coarse powder through a grinding machine. Then, 200 g of dried leaf powder was taken for Soxhlet extraction with 250 mL of methanol (99.98%) at boiling temperature, 64.7°C. The extract was concentrated with a rotary evaporator at 40 °C to provide a semi solid mass, designated as a methanol crude extract of leaf of *C. resiniferum*. The process was repeated for several times to increase the amount of crude extract. The resulting crude extract of *C. resiniferum* leaves was subjected for solvent-solvent partitioning into hexane (HFCR), chloroform (CFCR) and aqueous (AFCR) soluble fractions.

Preliminary phytochemical screening

Various solvent fractions (HFCR, CFCR and AFCR) of the crude methanol extract of *C. resiniferum* leaf were analyzed to confirm the phytochemical nature by qualitative tests.[11]

In vitro thrombolytic effect

Thrombolytic effect was evaluated following a standard method in which streptokinase (SK) was used as standard.[12] Briefly, 15 mL of venous blood was drawn from healthy volunteers without any recent history of oral contraceptive and anticoagulant therapy. The collected blood was immediately distributed into 27 previously weighed sterile alpine tubes (0.5 mL/tube) and the tubes were incubated at 37ºC for 45 min to form clots. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube-weight of tube alone). Plant samples solutions (500 µg/100 µL) were added separately to each alpine tube containing pre-weighed clot. As a standard, 100 µl of streptokinase, SK (15,00,000 I.U.) and as a control, 100 µL of methanol, hexane and chloroform were separately added to the control tubes. All the tubes were then kept at 37ºC for 90 min and observed for clot lysis. After incubation, the fluid released was removed carefully and tubes were again weighed to observe the difference in weight after clot disruption. The difference in weight taken before and after clot lysis was expressed as percentage of clot lysis.

$$\%\ \text{Clot lysis} = \frac{\text{Weight of the clot after lysis by plant sample}}{\text{Weight of the clot before lysis by plant sample}} \times 100\%$$

Anti-inflammatory activity

Protein denaturation inhibition assay

Protein denaturation inhibition assay[11] was employed to determine the anti-inflammatory effect of the *C. resiniferum* extractives using aspirin as standard. Sterile centrifuge tubes were taken and marked accordingly, as mentioned above. Then, 1.0 mL of 5% egg albumin solution and 2.8 mL phosphate buffer (pH 6.4 ± 0.2) were added to all test tubes. Aspirin (0.1 mg), Tween-80 or plant sample (500 µg/mL of Tween-80) was added as positive- and negative controls and test group, respectively. All the reaction mixtures after pH (5.6 ± 0.2) adjustment with 1.0N HCl were heated at 57 °C for 20 min. After cooling and filtering (Whatmann filter paper) the absorbance of each reaction mixture was measured at 660 nm using a UV-visible spectrophotometer (Shimadzu-1800). The test was repeated three times. Anti-inflammatory activity of each test sample was estimated by measuring the absorbance of the treatment groups and converting it into inhibition of albumin protein denaturation. The percent inhibition of protein denaturation was calculated by using the following formula:

$$\%\ \text{inhibition of protein denaturation} = \frac{A_{control} - A_{test}}{A_{control}} \times 100\%$$

Here, A = Absorbance for respective group.

Membrane stabilization assay

The method of Shinde *et al*[12] was used for the membrane stabilizing activity of plant extractives using human erythrocytes. Sterile centrifuge tubes (three for aspirin standard/positive control, three for negative control and three for each plant sample) were taken and marked accordingly. Then, 1.0 mL phosphate buffer (pH 7.4 ± 0.2), 2.0 mL hyposaline and 0.5 mL of erythrocyte suspension were added to all tubes. Then 1.0 mL of aspirin (0.1 mg) was mixed for standard group, 1.0 mL of distilled water was added to the control group tubes while 1.0 mL of plant samples (500 µg/mL) was mixed to the test group as marked. All the reaction mixtures were incubated at 37ºC in a Bio-Oxygen Demand incubator for 30 min and then centrifuged (10 min at 3000 rpm). The absorbance of the supernatants was measured using a UV-visible spectrophotometer (Shimadzu-1800) at 560 nm. The test was repeated three times for each plant extract. Membrane stabilization activity was determined by measuring the inhibition of hemolysis as follows.

$$\%\ \text{inhibition of hemolysis} = \frac{A_{control} - A_{test}}{A_{control}} \times 100\%$$

Experimental animals

Swiss mice (20-25 g) of either sex were procured from Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram, Bangladesh. The animals were kept under standard laboratory condition of room temperature (26 ± 2^{o}C) with 12 h light/dark cycle for 1 week to adjust the laboratory conditions before starting the experiment.[13] All experiments were performed according to the guidelines approved by the institutional ethical committee (2019-02-11/SUB/A-ERC/001).

Determination of antipyretic activity

The antipyretic activity of *C. resiniferum* was evaluated on Swiss mice by the method described by Muhammad *et al.*[14] The animals were divided into five groups, each group containing three mice. The normal body temperature of each mouse was recorded and 20% aqueous suspension of brewer's yeast (10 mL/kg) was injected subcutaneously in each mouse to induce pyrexia. After 17 h of Brewer's yeast injection, the rectal temperature of each mouse was measured using a digital thermometer. Only animals that showed an increase in temperature of at least 1.33 ºF were used for the study. The temperature was measured periodically at 1st, 2nd and 3rd h following the oral administration of standard paracetamol (150 mg/kg), normal saline (10 mL/kg) and the plant extracts (500 mg/kg). Antipyretic activity of the plant sample was measured by determining the rectal temperature of the mice of the test groups in comparison to the negative control and standard paracetamol group.

Analgesic activity

Central analgesic activity by tail immersion assay

Central analgesic activity of *C. resiniferum* was evaluated by tail immersion assay[15] in Swiss mice. Briefly, the experimental animals were randomly divided as mentioned above and received the plant extractives (500 mg/kg) by oral route. After treatment, at zero-hour, 1-2 cm of the tail of mice was immersed in warm water kept constant at 55° C. The time (in sec) taken for the mouse to flick its tail was regarded as the pain reaction time (PRT). The latency period was set as 20 s to avoid injury to mice. Following the administration of the test samples, pain reaction time was recorded at 0, 30, 60 and 90 min. Finally, the percentage of time elongation of tail immersion was estimated in respect to standard morphine (2 mg/kg) by the equation below.

$$\%\ \text{time elongation} = \frac{T_{test} - T_{control}}{T_{control}} \times 100\%$$

Where, T_{test} = Pain reaction time for each group.

Peripheral analgesic activity by formalin-induced pain

The peripheral analgesic activity was evaluated by formalin-induced licking and biting responses test.[15] Like tail immersion test, both standard aspirin (50 mg/kg) and plant samples (500 mg/kg) were administered to the experimental animals by oral route. Pain sensation was induced by subcutaneous injection of 1% formalin into the right hind paw of the experimental animals. As long as the animals feel pain, they continue to give licking and biting responses. Each licking and biting responses are counted and taken as an indication of pain sensation. Thirty minutes after formalin injection, number of licking and biting responses was recorded for 5 min for each mouse. The plant

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sample possessing analgesic activity will decrease the number of licking and biting responses in animal. The percent inhibition of formalin-induced licking in each treated group was then calculated by using the following equation:

$$\%\ \text{inhibition of licking response} = \frac{N_{control} - N_{test}}{N_{control}} \times 100\%$$

Where, N = Mean number of licking and biting responses for each group.

Hypoglycemic activity

Hypoglycemic effect of plant extracts on Swiss mice was checked by oral glucose tolerance test.[16] The animals were randomly divided into control, standard and test groups containing three mice in each group. Each group received a particular treatment. At zero-hour, test samples (500 mg/kg), negative control (1% Tween 80) and glibenclamide (10 mg/kg) were administered orally to the respective mice group by means of oral gavage tube. After 60 min, all animals were treated with 10% glucose solution (2 mg/kg). Blood glucose level was recorded by glucometer after 30, 60 and 120 min of glucose loading in the mice.

Statistical analysis

For all biological studies, three replicates of each test sample or three mice in each group were used for statistical comparison and analysis, and the values were presented as mean ± SEM.

Results and Discussion

Phytochemical study

Phytochemical study was performed to analyze the chemical constituents present in the hexane, chloroform and aqueous soluble fractions of methanol extract of *C. resiniferum* leaf. The preliminary phytochemical investigation revealed the presence of plant constituents (Table 1) which are known to exhibit important pharmacological actions. Alkaloids, saponins and amides were detected in all the solvent fractions of *C. resiniferum* leaf extract, but flavonoids were absent. Alkaloids, glycoside, steroids, reducing sugar, amides, saponins were present in the chloroform and aqueous fractions. Both hexane and chloroform fractions contain alkaloids, tannins, saponins amides. The results of the preliminary phytochemical investigation laid the foundation for more experimental works on *C. resiniferum*.

Thrombolytic activity

Previously, several research works have been carried out to discover the natural sources having thrombolytic effect. Because, thrombolytic agents of natural origin are safe and effective in the management of cardiovascular diseases including myocardial infarction, strokes and deep vein thrombosis. There are numerous examples of Bangladeshi medicinal plants having thrombolytic effects.[17] To find out plant-based cardioprotective drugs, the extractives of *C. resiniferum* were assessed for *in vitro* thrombolytic activity using human RBC and the results are presented in Figure 1. The chloroform, hexane and aqueous soluble fractions of methanol extract of *C. resiniferum* leaf at the dose of 500 µg/100 µl showed prominent thrombolytic activity by clot lysis of 57.32%, 42.85% and 34.08%, respectively. The standard streptokinase caused 80.1% lysis of clot while the distilled water (served as a negative control) showed negligible percentages of lysis of clot (4.16%). Previous study confirmed that tannins, alkaloids and saponins demonstrated significant clot lysis activity through the breakdown of fibrinogen and fibrin in a clot. The results of our phytochemical analysis revealed that different solvent fractions of *C. resiniferum* leaf extracts possess tannin, alkaloid and saponin which could be associated with its observed clot lysis activity in the study.[18]

Anti-inflammatory activity

Protein denaturation inhibition assay

Inflammation is a pathological state that is associated with a wide range of ailments such as arthritis, diabetes, obesity, cardiovascular disease etc. Commonly prescribed synthetic anti-inflammatory agents such as NSAIDs and steroids are related with adverse effects, for example gastrointestinal upset, gastric ulcer, bleeding, liver damage etc.[19] Due to these toxic effects, the demand for development of safe and effective anti-inflammatory agent from plant sources is rising globally. Therefore, in order to find out natural anti-inflammatory agent, we have selected an endangered medicinal plant *C. resiniferum* for preliminary screening of its anti-inflammatory potential by egg albumin denaturation inhibition assay and RBC membrane protection assay. The test extract and fractions of *C. resiniferum* (500 µg/mL) inhibited albumin denaturation and hemolysis of human RBCs. Protein denaturation inhibition assay is a common method for the assessment of anti-inflammatory potential of plant extracts. Protein (egg albumin) denaturation stimulates the production of auto-antigens which is associated with the pathogenesis of certain inflammatory diseases such as arthritis.[20] Substances that can inhibit protein denaturation or protect the RBC membrane against harmful chemicals, therefore, would be probable candidate for anti-inflammatory drug development. During inhibition of protein denaturation assay, the chloroform fraction at the dose of 500 µg/mL showed the highest inhibitory capacity with 47.08%, followed by hexane (39.96%) and aqueous fractions (35.29%) of *C. resiniferum*, while the standard aspirin exhibited 77.10% inhibition of albumin denaturation (Figure 2A).

RBC membrane stabilizing assay

Anti-inflammatory activity of *C. resiniferum* extractives was further established by the results (Figure 2B) of human RBC membrane stabilization assay.[21] The ability of all the solvent fractions of methanol extract of *C. resiniferum* leaf to protect the RBC membrane against hypotonic solution was found to be statistically significant ($P<0.01$). The highest inhibition of hypotonic solution induced RBC hemolysis was displayed by the chloroform fraction (49.68%). However, in both cases none of the control group (NC-negative control; HC-hexane control; CC-chloroform control) had any inhibitory effect, suggesting that only the plant constituents in the test samples produced anti-inflammatory effect. The breakdown of RBC membrane facilitates the release of lysosomal content into cytosol, which can initiate inflammatory response at the site of tissue injury.[21] The results (Figure 2A, 2B) suggested that the plant extracts could have remarkable anti-inflammatory potential like NSAIDs via inhibition of albumin denaturation and membrane protecting mechanism.

Table 1: Phytochemical constituents found in hexane, chloroform and aqueous fraction of methanol extract of *C. resiniferum* leaves.

Phytochemical	Plant sample		
	Hexane fraction	Chloroform fraction	Aqueous fraction
Alkaloids	+	+	+
Glycosides	-	+	+
Steroids	-	+	+
Tannins	+	+	-
Flavonoids	-	-	-
Saponins	+	+	+
Reducing sugars	-	+	+
Gums	+	-	+
Amides	+	+	+

'+' = present, '-'= absent.

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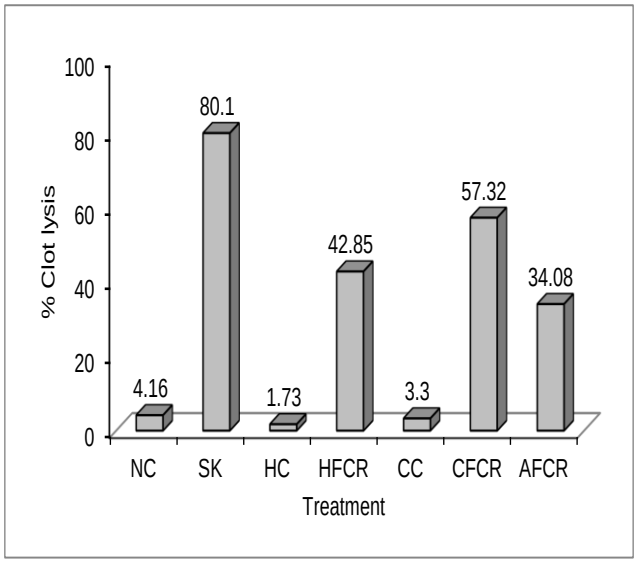

Figure 1: Thrombolytic activity (in terms of % clot lysis) of hexane, chloroform and aqueous fractions of methanol extract of *C. resiniferum* leaves.
NC = Negative control; SK = Streptokinase; HC = Hexane control; HFCR = Hexane fraction of methanol extract of *C. resiniferum* leaf; CC = Chloroform control; CFCR = Chloroform fraction of methanol extract of *C. resiniferum* leaf; AFCR = Aqueous fraction of methanol extract of *C. resiniferum* leaf.

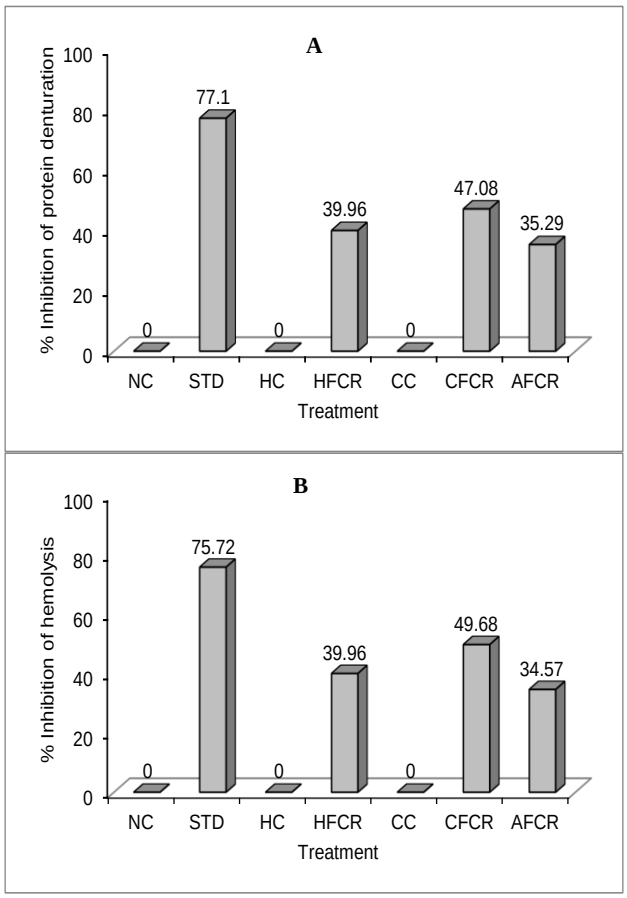

Figure 2: Anti-inflammatory activity of *C. resiniferum* extractives by protein denaturation assay (A) and by human RBC membrane stabilizing assay (B). STD = Standard aspirin.

Antipyretic activity

It is well known that an inflammatory response is connected with numerous manifestations such as high body temperature and pain. Therefore, a compound having anti-inflammatory potential may also show antipyretic and analgesic properties. Brewer's yeast-induced hyperthermia is a useful method[22] to evaluate the antipyretic effect of plant extracts. Subcutaneous injection of yeast cell induces pyrexia, also called pathogenic fever via the enhanced production of prostaglandin through COX-2 expression.

Therefore, the inhibition of prostaglandin production by blocking the COX-2 enzyme could be the probable mechanism of antipyretic action as that of paracetamol.[23] In the antipyretic activity assay, the oral administration of the test samples significantly reduced the rectal temperature of the febrile mice induced by yeast. The antipyretic action of the test sample was prominent which persists up to 3h after extract administration as shown in Table 2. Among the extractives, the highest antipyretic effect was noticed for the chloroform fraction of *C. resiniferum* which downregulated the body temperature to 98.8 ± 0.04 °F, while 99.6 ± 0.23 °F was observed in paracetamol treated group at 3rd h of the experiment. Reduction of high body temperature might be due to the amalgamation of anti-inflammatory and antipyretic actions of *C. resiniferum* via the inhibition of prostaglandin production. However, further studies are required to confirm the mechanism of antipyretic activity of the tested extracts.

Analgesic activity

Central analgesic activity

Analgesic compounds are being searched from natural sources as substitutes to synthetic drugs because of their natural origin and little adverse effect.[24] Analgesic activity of *C. resiniferum* extractives was determined by tail immersion method as well as by formalin-induced licking and biting response model in mice. In tail immersion method, the hexane fraction and reference drug morphine significantly increased the PRT (pain reaction time) with 228.57% and 442.86% elongation, respectively at 90 min after the oral administration of plant sample in mice (Table 3). The chloroform fraction prolonged the PRT with 236.36% elongation at 60 min, after that its effect started to diminish at the level of 42.86% at 90 min after extract administration. The results of tail immersion model indicated that the plant extracts possess moderate analgesic effect in the experimental mice.

Peripheral analgesic activity

Peripheral analgesic activity was evaluated by formalin test in mice model. Subcutaneous injection of formalin elicits the biogenesis of inflammatory mediators including PGE_2, PGI_2 etc., which ultimately led to induce inflammation in treated animals.[25] As long as the animals feel pain, they continue to give licking and biting responses which are taken as an indication of pain sensation. Any substance having pain relieving ability is supposed to diminish the number of licking and biting responses of animals. At the dose of 500 mg/kg, the chloroform fraction of *C. resiniferum* showed maximum 29.82% inhibition of formalin-induced licking and biting responses, followed by aqueous fraction (25.38% inhibition) and hexane fraction (23.72% inhibition) (Table 4). The standard aspirin exhibited 53.22% inhibition at a dose of 50 mg/kg. The plant extract, like centrally acting morphine drug, produced a repressing effect on the nociceptive response in tail-immersion assay. The test extractives of *C. resiniferum* also inhibited formalin-induced licking and biting responses in mice. Therefore, it can be suggested that the analgesic effect of *C. resiniferum* is also peripherally mediated. The phytochemical screening revealed that the *C. resiniferum* extractives contain bioactive components including alkaloids, glycoside, steroids, reducing sugar, amides, saponins. Many researchers have reported the anti-inflammatory action of triterpenoid saponins,[26] glycosides[27] and alkaloids.[28] Therefore, it seems that analgesic, anti-inflammatory and antipyretic profile of C. *resiniferum* might be due to the presence of triterpenoids, glycosides and alkaloids in the extracts. However, further laboratory studies are required to confirm the credible mechanism.

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Table 2: Antipyretic effect of hexane, chloroform and aqueous fraction of methanol extract of *C. resiniferum* leaves on yeast-induced pyrexia in mice.

Treatment	Dose	Rectal temperature in ºF			
		After administration of plant sample/drug			
		0 h	1 h	2 h	3 h
Negative control	10 mL/kg	101.8 ± 0.04	101.5 ± 0.04	101.3 ± 0.04	101.2 ± 0.04
Paracetamol	150 mg/kg	103.0 ± 0.02**	101.2 ± 0.006*	100 ± 0.07*	99.6 ± 0.23**
Hexane fraction	500 mg/kg	101.5 ± 0.19**	101.0 ± 0.04**	99.0 ± 0.04***	99.6 ± 0.08**
Chloroform fraction	500 mg/kg	101.6 ± 0.07**	100.7 ± 0.04**	99.5 ± 0.07**	98.8 ± 0.04**
Aqueous fraction	500 mg/kg	101.4 ± 0.04*	100.6 ± 0.04*	99.6 ± 0.33*	99.4 ± 0.11*

*P < 0.05, **P < 0.01, values are expressed as mean ± Standard error (n = 3)

Table 3: Analgesic effect of hexane, chloroform and aqueous fraction of methanol extract of *C. resiniferum* leaves by tail immersion method in mice.

Treatment	Dose	% Elongation		
		Time after administration of plant sample/drug		
		30 min	60 min	90 min
Negative control	30 mL/kg	-	-	-
Morphine	2 mg/kg	333.33	354.55	442.86
Hexane fraction	500 mg/kg	208.33	45.45	228.57
Chloroform fraction	500 mg/kg	50.0	236.36	42.86
Aqueous fraction	500 mg/kg	33.33	36.36	36.36

Hypoglycemic effect

Medicinal plants are the immense sources of the bioactive lead targets that might set up a strong basis for the development of new antidiabetic agent. For example, metformin was discovered after establishing the hypoglycemic effect of its structurally related compound galegine, an alkaloid that was isolated from *Galega officinialis*.[29] Due to economic cost, easy availability and less side effects, there has been raising demand for the use of plant-based medicines with anti-diabetic activity. Therefore, the scientists have been exploring the plant sources to find out the bioactive compound with glucose lowering potential. In the oral glucose tolerance test, the glucose loaded diabetic mice were treated by *C. resiniferum* extractives. The obtained blood glucose level (mmol/L) for plant extracts and control groups were presented in Table 5. All the plant extracts (500 mg/kg) reduced blood glucose level in 0, 30, 60, 120 min compared to the control group. This effect was persistent up to 120 min after the oral administration of glucose. The hypoglycemic effect of *C. resiniferum* might be associated with the presence of bioactive phytochemicals having antidiabetic effects via the stimulation of insulin release by β-cells or inhibition of glucose absorption from intestine.[30] Further biological investigations are needed to isolate the compound responsible for this observed hypoglycemic action.

Table 4: Analgesic effect of hexane, chloroform and aqueous fraction of methanol extract of *C. resiniferum* leaves by formalin-induced licking and biting response method in mice

Treatment	Dose	Licking and biting responses (s)			Average (s)	Inhibition (%)	t-test
		M-1	M-2	M-3			
Negative control	0.1 mL/10 g	290	300	265	285.0	0	--
ASA	50 mg/kg	184	101	115	133.33	53.22	4.48 < 0.05
Hexane fraction	500 mg/kg	225	218	209	217.33	23.72	4.29 < 0.05
Chloroform fraction	500 mg/kg	205	201	194	200.0	29.82	5.25 < 0.05
Aqueous fraction	500 mg/kg	220	212	206	212.67	25.38	6.54 < 0.05

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Table 5: Hypoglycemic effect of hexane, chloroform and aqueous fraction of methanol extract of *C. resiniferum* leaves by oral glucose tolerance test in mice.

Treatment	Dose	Mean plasma level of glucose in mmol/L			
		Time after administration of plant sample/drug			
		0 min	30 min	60 min	120 min
Negative control	0.1 mL/kg	5.9	6.6	7.3	5.5
Glibenclamide	10 mg/kg	4.2	3.8	3.5	3.4
Hexane fraction	500 mg/kg	4.9	4.6	4.2	3.9
Chloroform fraction	500 mg/kg	4.9	4.7	4.4	4.1
Aqueous fraction	500 mg/kg	5.0	4.6	4.1	3.9

Conclusion

Our study demonstrated that the plant has potential *in vitro* and *in vivo* pharmacological properties which authenticate its traditional uses in Bangladeshi tribal people. The chloroform fraction of methanol extract of *C. resiniferum* leaf showed promising thrombolytic, anti-inflammatory and analgesic effect. The results suggest that *C. resiniferum* can be a potential source for bioactivities. Nonetheless, further study is necessary to discover specific bioactive compounds present in the tested plant samples and their mechanism of action.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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