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In vitro Anti-inflammatory, Antioxidant and Membrane Stabilization Activities of Leaf Extracts of *Byttneria pilosa* Roxb.

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Abstract

This current research work was conducted an investigation into the biological activities of various fractions of *Byttneria pilosa* Roxb. (Family: Sterculiaceae) and analyze the chemical compositions. Preliminary examination of the raw fractional extractives identified the presence of alkaloids, glycosides, flavonoids, saponins, reducing sugars, and tannins. The standard was acetyl salicylic acid for heat-induced method to evaluate the anti-inflammatory and membrane-stabilizing properties. The crude ethanol extract displayed a moderate activity, inhibiting albumin denaturation by 44.7% compared to the positive control. All three extractives also demonstrated significant ($p<0.05$) *in-vitro* membrane-stabilizing activity. Additionally, the plant showcased robust antioxidant properties, as evidenced by the IC_{50} values of the ethanol extract, n-hexane extract and chloroform extracts (5.3, 5.4, and 6.2 µg/ml, respectively), one the contrary, the acetyl salicylic acid as standard drug exhibited an IC_{50} value of 3.8 µg/ml. In summary, the outcomes of this investigation propose that this specific plant species holds promise for the discovery of novel natural bioactive compounds.

Key words: *Byttneria pilosa*, phytochemicals, anti-inflammatory, antioxidant, membrane stabilization activity.

Introduction

Medicinal plants serve as a notable reservoir of naturally occurring compounds with medicinal properties, referred to as phytochemical constituents. Consequently, they possess significant value in the treatment of human diseases and play a pivotal role in the healing process (Sohani, 2021). Given the abundant availability of medicinal plants in rural and tribal areas of Bangladesh, these regions have long relied on plants and herbs as the primary means of treatment (Bardhan *et al.*, 2018). Historically, the plant *Byttneria pilosa* Roxb. has been employed in the treatment of conditions such as bone fractures, boils, scabies, rheumatism, snake bites, syphilis, elephantiasis, poisoning, and eye infections. According to scientific research, it has notable anti-inflammatory, analgesic, anti-diarrheal, anxiolytic, locomotor, sedative, and anti-obesity effects. In Bangladesh, this plant local name is 'Harjora' and it is used by the indigenous hill-tract population to alleviate various health conditions such as rheumatalgia, snake bites, syphilis, fractured bones, elephantiasis, and as an antidote for poisoning (Jyoti *et al.*, 2020). The stems of *B. pilosa* are crushed and then employed for treating conditions such as boils, rheumatism, snake bites, and syphilis. The Khumi community in Bangladesh uses a paste produced from the delicate stem to treat raptured bones. In the Tripura community, elephantiasis is treated by applying a root paste to the afflicted areas. Additionally, root juice acts as a poisoning counteragent. The plant also yields froth that irritates

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the eyes. Using physicochemical analysis and Fourier-transform infrared spectroscopy (FTIR) analysis, a substance called beta-sitosterol was extracted from the roots of *B. pilosa*. The forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Sylhet, Srimangal, Gajni (Sherpur), and Habiganj districts in Bangladesh are the main habitats for *B. pilosa*. This plant is characterized as a glabrous herb that completes its life cycle within a year, making it suitable for annual cultivation. It reproduces through seeds and reaches maturity during the winter season (Sikder *et al*., 2022).

Despite the widespread recognition and medicinal significance of this plant, there remains a notable absence of scientific validation regarding its phytochemical and pharmacological aspects. Consequently, our objective was to assess the ethanol extract of *B. pilosa* leaves for its potential anti-inflammatory, anti-oxidant and membrane stabilization activities.

Material and Methods

Plant material collection and preparation of drug material: On March, 2020 the fresh plant leaves were collected from the hilly areas of Sitakund, Chattogram, Bangladesh (GPS coordinates: 22° 36′ 11.16″ N, 91° 40′ 38.64″ E). The plat species was identified by the taxonomist of Bangladesh Forest Research Institute, Chattogram and a sample of herbarium was preserved with an accession number. These collected leaves underwent a series of procedures. Initially, any unnecessary or unwanted portions were eliminated. Rotten and dry leaves were discarded. Through the use of a fan-generated air flow, sieving was employed to separate the soil and dust from the leaves. Afterward, the leaves were dried in a shaded area where the temperature remained below 50°C (Jyoti *et al.*, 2020). Following the drying process, they were crushed into a course powder using a grinder. An airtight container was used to preserve the powder and a cool and dark environment was chosen to store this powder until the extraction process began.

Crude Extractions and fractionations: The crushed powder materials (120 g) were gathered in clean, amber-colored containers with a capacity of 3 liters and soaked in ethanol (1L) for duration of 20 days, during which it was periodically shaken and stirred. Taking after this soaking period, the crude ethanolic fraction was attained by filtering the entire extractive mixture through a clean cotton plug and Whatman No. 1 filter paper. The solution obtained was at that point subjected to a rotary evaporator, keeping up a low temperature (not exceeding 40°C) and pressure. The concentrated crude extract (20 gm) was carefully stored for future use. To perform solvent-solvent partitioning, the Kupchan method outlined by Van Wagenen *et al*., (1993) was applied. The ethanolic leaf extract (15 g) resulting from this process was dissolved in 10% aqueous ethanol and subsequently subjected to progressive extraction with n-hexane and chloroform. The resulting extracts were collected, and the solvent was evaporated at a temperature below 50°C. This evaporation process led to the formation of a gummy concentrate. The weight of each gummy concentrate was measured, and it was then transferred to a properly labelled, clean, airtight container, which was stored at a temperature of 4°C

Qualitative screening of phytoconstituents: All residual chemicals were of analytical grade. The reagents used in the experiments include Fehling's solution A, Fehling's solution B, Benedict's reagent, Salkowski reagent, Libermann-burchared reagent, 5% Ferric chloride solution, 10% Potassium dichromate solution, Keller-Kiliani reagent, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Hager's reagent and Molisch's reagent . The crude and fractions were qualitatively examined for alkaloid, steroid, tannin, saponin, glycoside, reducing sugar, gum, amide, and flavonoid (Faruq *et al*., 2017).

Screening for anti-inflammatory activity: The anti-inflammation property was tested through minor modifications to the method proposed by Mizushima and Kobayashi (1968). Here the control group's *in vitro* suppression of protein cleavage was compared with the positive control and test groups. The anti-inflammatory activity was directly correlates to the

degree of protein denaturation and the higher inflammation would result in higher measured absorbance. It is noted that 15 clean and dry centrifuge tubes were utilized in the experiment. The standard, the control, had a set number of three tubes apiece along with the other nine tubes for each extract. 1ml of a 5% egg albumin solution was added to each treatment tube, while 2ml of double-distilled water (DDW) was added to each control tube. The test groups received 2 ml of the designated test extracts, whereas 2 ml of acetylsalicylic acid with a necessary amount was given to the positive control groups. To ensure uniformity, 1N HCl was used to bring the pH of each reaction mixture to 5.6 ± 0.2. The reaction mixtures were then heated for five minutes at a temperature of 60°C. The Whatman filter paper was cooled and filtered and then the absorbance was measured at 660 nm by a spectrophotometer. For each extract, this procedure was performed three times.

Evaluation of anti-oxidant activity: This qualitative and quantitative test was done to find out the oxidation potential of the plant species. Thin layer chromatography (TLC) assessed the qualitative antioxidant property. The diluted solution was spotted on silica gel TLC plates and a variable polarities of solvent systems was prepared to separate polar and non-polar constituents of the extract. The dried plates were then sprayed with 0.004% DPPH (1, 1-diphenyl-2-picryl hydrazyl) in ethanol. The bleaching of DPPH by the resolved bands was examined for 10 minutes, and any color changes (yellow on purple background) were recorded (Matkowski, 2006).

To evaluate quantitative DPPH radical scavenging activities, 0.1 ml of ethanol, chloroform, and n-hexane extracts at various concentrations (10, 20, 30, 40, and 50 µg/ml) were added to 3 ml of a 0.004% ethanol solution of $DPPH^{\cdot}$. The reaction tubes were kept in the dark, except for the control tubes, for a duration of 30 minutes. After 30 minutes, the absorbance of the resulting solution was measured at 517 nm against a blank.

The rate of DPPH radical scavenging activity (%SCV) was calculated by comparing the experimental results with the control (not treated with extract) using the following formula:

$$\% \text{ SCV} = (A_0 - A_1) / A_0 \times 100$$

Where:

SCV = Free radical scavenging activity,

A_0 = Absorbance of the control, and

A_1 = Absorbance of the test (extracts/standard).

About 50% inhibition (IC_{50}) value obtained from the result of the extract concentrations were determined from the graph plotted as % SCV versus concentration curve. The test was done two times, where the standard was the ascorbic acid.

Membrane stabilization activity: The standard solution was made by dissolving 50 mg of acetyl salicylic acid in 100 ml of double distilled water (DDW), which resulted in an initial concentration of 500 µg/ml. To get concentrations of 250 µg/ml and 125 µg/ml, this solution was further diluted.

One hundred ml of DDW was combined with 900 mg and 500 mg of NaCl to create isotonic and hypotonic solutions, respectively. Furthermore, 5ml of whole blood were drawn into a tube from human volunteers who were in good health. To create a 10% suspension, the red blood cells (RBCs) were diluted using RBC diluting fluid up to 20 ml in 1 ml of fresh whole human blood. 2ml of this solution were then further diluted with18 ml of regular saline solution to obtain the necessary 10% RBC suspension. 50 mg of each of the different crude fractions were mixed with 100 ml of the corresponding solvents—ethanol, n-hexane, and chloroform—to make the stock solution for the test groups. The stock solution was thereafter further diluted as needed.

Statistical Analysis: The values were presented in mean ± SEM (standard error mean), where $^{a}p<0.05$ and $^{b}p<0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance ANOVA (Dunnett's test) compared to the control groups.

Results and Discussion

Phytochemical screening: The crude ethanol extract of *B. pillosa* included alkaloid, glycoside,

tannin, flavonoid, saponin, and reducing sugar, according to phytochemical analysis. Alkaloid, saponin, and reducing sugar were all present in chloroform extract. On the other hand, alkaloid, glycoside, tannin, flavonoid, and saponin were also present in the n-hexane extract (Table 1).

Anti-inflammatory activity: Test results in the Table 1 revealed that at a concentration of 500 µg/ml, the crude ethanol extracts inhibited inflammation by 44.77%, exhibiting a moderate degree of activity. The anti-inflammatory properties of the chloroform soluble fraction were very weak, with just 15.04% inhibition of inflammation, whereas the n-hexane extract showed a moderate degree of action with a 31.37% inhibition. As indicated in Table 2, these results were compared to the common medication Acetyl salicylic acid (ASA). The anti-inflammatory efficacy was assessed from the absorbance of the treatment groups which was followed by converting the data into total inhibition of protein denaturation.

Anti-oxidant activity: The antioxidant test revealed that, the ethanol extract had the highest DPPH inhibitory effect of 88.6%, followed by the chloroform extract with 75.9% inhibition, and the n-hexane extract with 61.1% inhibition (Table 3). The IC50 values, which represent the concentration required for 50% inhibition, were determined for each extract: 5.3 µg/ml for ethanol, 5.4 µg/ml for chloroform, and 6.2 µg/ml for n-hexane. In comparison, the IC50 value for Ascorbic acid, a standard antioxidant, was found to be 3.8 µg/ml (Table 4). These test results provide strong evidence of the antioxidant potential of the *B. pilosa* extracts.

Table 1. Phyto constituents present in *B. pilosa.*

Chemical group	Alkaloid	Glycoside	Steroid	Tannin	Flavonoid	Saponin	Reducing sugar	Gum	Amide
Crude ethanol fraction	+	+	-	+	+	+	+	-	-
n-Hexane soluble fraction	+	+	-	+	+	+	-	-	-
Chloroform soluble fraction	+	-	-	-	-	+	+	-	-

Table 2. Anti-inflammatory activities of different treatment groups of *B. pilosa.*

Test groups	Dose	% IPD (mean ± SEM)
Control (DDW)	---	00.00
Standard (ASA)	500 µg/ml	72.04[a]±0.002
	250 µg/ml	51.75 [a]±0.006
	125 µg/ml	32.37[b]±0.001
Crude ethanol fraction of *B. pilosa*	500 µg/ml	44.77[a]±0.008
	250 µg/ml	38.24[a]±0.002
	125 µg/ml	29.08[a]±0.003
n-Hexane soluble fraction of *B. pilosa*	500 µg/ml	15.06[b]±0.001
	250 µg/ml	13.15[a]±0.002
	125 µg/ml	10.68[b]±0.001
Chloroform soluble fraction of *B. pilosa*	500 µg/ml	31.37[b]±0.002
	250 µg/ml	22.39[a]±0.001
	125 µg/ml	20.22[b]±0.003

SEM = Standard Error of Mean ; % IPD = % Inhibition of protein denaturation; DDW = Doubled distilled water; [a]$p<0.05$; [b]$p<0.001$.

Table 3. DPPH free radical scavenging activities of the plant extractives.

Concentration (µg/ml)	% inhibition of ascorbic acid	% inhibition of ethanol extract	% inhibition of chloroform extract	% inhibition of *n*-hexane extract
100	91.0	88.6	75.9	61.1
80	87.7	84.3	64.6	53.9
60	83.1	87.4	57.7	47.2
40	76.5	73.5	53.8	41.5
20	72.6	65.6	49.4	33.5

Table 4. IC50 values of different fractions and standard drug.

Treatment groups	IC_{50} (µg/ml)
Ascorbic Acid	3.8
Crude ethanol fraction	5.3
n-Hexane soluble fraction	6.2
Chloroform soluble fraction	5.4

Table 5. Membrane stabilization activities of *B. pilosa*.

Test groups	Dose (µg/ml)	Inhibition of haemolysis (%) ± SEM
Control	---	---
Positive control (Acetyl Salicylic Acid)	500	69.99 ± 0.0040 [a]
	250	60.05 ± 0.0040 [a]
	125	47.09 ± 0.0080 [a]
Crude ethanol fraction of *B. pilosa*	500	47.09 ± 0.0004 [b]
	250	24.03 ± 0.0007 [b]
	125	12.87 ± 0.0070 [a]
n-Hexane soluble fraction of *B. pilosa*	500	22.15 ± 0.0040 [a]
	250	11.55 ± 0.0060 [a]
	125	4.50 ± 0.0200 [a]
Chloroform soluble fraction of *B. pilosa*	500	12.01 ± 0.0130 [a]
	250	7.53 ± 0.0010 [a]
	125	4.71 ± 0.0040 [b]

SEM = Standard Error of Mean; [a]$p<0.05$; [b]$p<0.001$.

Membrane stabilization activity: The experiment showed the effects of different extracts (ethanol, n-hexane, and chloroform) on the heat-induced total haemolysis of red blood cells (RBCs). At a higher dose of 500 µg/ml, the ethanol extract inhibited haemolysis by 47.09%, while the n-hexane and chloroform extracts inhibited it by 22.15% and 12.01% respectively. Acetyl salicylic acid (ASA), a common medication, decreased hemolysis by 69.99% at the same dose. These findings suggest that the plant's ethanol extract had moderate amount of membrane stabilization action, however, the n-hexane and chloroform extracts had less activity in comparison to the conventional medication (Table 5).

Conclusion

A systematic investigation evaluated the phytochemicals and biological potentials of ethanol, n-Hexane and chloroform fractional extracts of *Byttneria pilosa* Roxb. leaves (Family: Sterculiaceae) in this study. Compounds like alkaloid, glycoside, tannin, flavonoid, saponin, and reducing sugar were all found by qualitative phytochemical analysis. The crude ethanol extract displayed moderate anti-inflammatory activity while the n-hexane and chloroform extracts showed mild and very poor activity, respectively. The ethanol extract moderately stabilize the membrane, while the n-hexane and chloroform extracts demonstrated exhibited low activity compared to standard drugs. In the antioxidant test, the ethanol extract exhibited potent antioxidant activity in comparison to the standard, while the chloroform and n-hexane extracts showed moderate activity. Overall, these findings indicate that the extracts of B. pilosa possess diverse phytochemical constituents and demonstrate promising biological activities, including anti-inflammatory, membrane stabilization, and antioxidant properties. These results highlight the potential of this plant species for the discovery of new bioactive compounds.

Declaration of interest

The authors declare no conflict of interest

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