Phytochemical and pharmacological investigation of the ethanol extract of *Byttneria pilosa* Roxb.

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**Abstract**

**Background:** Traditionally, the herb *Byttneria pilosa* Roxb. is used for bone fractures, boils, scabies, rheumatalgia, snake bites, syphilis, elephantiasis, poisoning, and eye infection. Scientific reports suggest that it has significant anti-inflammatory, analgesic, anti-diarrheal, anxiolytic, locomotion, sedative and anti-obesity effects. This study aims at the investigation of the phytochemical and pharmacological properties of the ethanol extract of this herb.

**Methods:** Fresh whole plant was extracted with absolute ethanol. A preliminary phytochemical investigation was followed by the evaluation of thrombolytic, anti-inflammatory, and anti-nociceptive activities by applying human clotted blood lysis, egg albumin, and acetic acid-induced writhing models, respectively.

**Results:** Phytochemical investigation suggests that *B. pilosa* possesses alkaloids, flavonoids, glycosides, terpenoids, tannins, saponins, and reducing sugars. The extract exhibited clot lysis and anti-inflammatory effects in a concentration-dependent manner. *B. pilosa* extract at 250 and 500 mg/kg also showed significant (*p* < 0.05) dose-dependent anti-nociceptive activity in Swiss albino mice.

**Conclusion:** The *B. pilosa* ethanol extract contains many important secondary metabolites and has thrombolytic, anti-inflammatory, and anti-nociceptive activities. More research is necessary on this hopeful medicinal herb.

**Keywords:** *Byttneria pilosa*, Anti-inflammatory, Anti-nociceptive, Clot lysis

**Introduction**

Medicinal plants still remain a potential source of modern therapeutic agents for various diseases [51]. It may be due to their low side effects and high safety profile in experimental animals and humans [13]. Plant-derived compounds are beneficial for designing new chemical entities in the drug discovery and development processes. More than 60–70% of the antimicrobial and anti-cancer agents currently in clinical use are natural products or their derivatives [34].

In the drug discovery and development context, the bioactivity of plants or their parts such as leaves, stems, and roots frequently used in traditional medicine is evaluated in laboratory-based bioassays [50]. In this context, plant-based lead compounds have become inspiration for the development of potential drugs [29]. Generally, medicinal plants or their derivatives are promising sources of alternative therapeutics [20] and can be used for some life-threatening diseases, including the novel coronavirus disease 2019 (Covid-19) [23].

*Byttneria pilosa* Roxb. (Malvaceae) (Fig. 1) is commonly known as Harjora in Bangladesh. It is found in the forests of Chittagong, Chittagong Hill Tracts, Cox’s Bazar, Sylhet, Srimangal, Gajni (Sherpur) and Habiganj districts of Bangladesh. It is an annual glabrous herb that can be grown as an annual, reproducing from the seeds [17]. The stems are large woody climbers with grooved, strigose, branchlets, and the leaves are sub or biclar, palmately 3-lobed, and pilose on both surfaces. The flowers of *B. pilosa* are minute campanulate, in a lax,
much branched inflorescence, while the capsule is globose, the size of a large cherry, and studded with subulate barbed prickles. It becomes mature in the winter season.

*B. pilosa* is used by rural people as a traditional medicine. The paste prepared from the tender stem with leaves is tied around limbs for the treatment of fractured bones. The crushed stems and an infusion of the leaves are used to treat boils and scabies, respectively. The root paste is used to treat elephantiasis [22]. It is also used for rheumatalgia, snake bite, syphilis, poisoning, and eye infection [62]. A recent study suggests that this herb has significant anti-inflammatory, analgesic, anti-diarrheal, anxiolytic, locomotion and sedative effects in various test systems [22]. The authors also performed molecular docking, PASS prediction, and ADME analysis that suggests beta-sitosterol might be a potential candidate for these biological activities.

Our study was aimed at screening the phytochemical and pharmacological activities of the ethanol extract of *B. pilosa*. The results suggest that *B. pilosa* ethanol extract contains many important secondary metabolites, including alkaloids, flavonoids, glycosides, terpenoids, tannins, and saponins. The extract showed clot lysis, anti-inflammatory, and anti-nociceptive activities in different test systems.

Plant-based molecular farming is cheap and relatively scalable [32]. Furthermore, they are generally safer because they do not pose the risk of product contamination with endotoxins, infectious viruses, or prions [46]. On the other hand, plants can perform complex posttranslational modifications (e.g., Asn-linked (N) glycosylation), that can be further engineered to achieve humanized biomolecules [12]. Our current findings suggest that *B. pilosa* herb may be a hopeful alternative phyto-based therapeutic tool to manage many diseases in humans. However, the conclusion of this project has been drawn on the basis of limited non-clinical and pre-clinical studies on its crude extract. The active principles and their possible molecular interactions are yet to be discovered.

It is clear that the knowledge of ancient and traditional systems of medicine has now transcended to the modern pharmaceutical industry. Therefore, the focus of any phyto-pharmacological study is often to discover new therapeutic agents or lead compounds from medicinal plants. However, appropriate selection of target plant species, in this case, plays an important role. Interestingly, serendipity has played a significant role in plant-based drug discovery. For instance, the discovery of dicoumarol from fatal cattle poisoning by *Melilotus officinalis* was simply a serendipitous discovery [28], which then led to the development of the well-known anticoagulant warfarin. Conducting phyto-pharmacological research without any working hypotheses may lead to such a type of unexpected discovery. However, the chances of success are much poorer than with any targeted approach, and for this reason, we often rely on the basis of ethnopharmacological evidence and extensive literature activities on medicinal plants. Moreover, challenges, in this case, also lie in devising appropriate methods besides selecting the right plants that may fulfill these criteria. Knowing the overall facts, we have identified *B. pilosa* by an expert and selected some in vitro and in vivo assay methods for this study. Prior to this, we also gathered knowledge about its traditional and scientific evidence from reliable and authentic sources.

**Materials and methods**

**Collection, identification, and extraction of plant materials**
The fresh whole plant was collected from the forest of the University of Chittagong, Chittagong, Bangladesh in the month of January and was identified by the taxonomist at the Bangladesh National Herbarium, Dhaka. A voucher specimen was deposited with the accession no.: DACB 46758. After collection, the plant materials were
shade dried (temperature not exceeding 45 °C) for 7 days. Then the dried materials were cut into small pieces and ground into coarse powder with the help of a suitable mechanical chopper. The powdered materials (250 g) were then soaked in 700 mL of absolute ethanol with occasional manual stirring for 15 days. Then the extract was finally filtered using Whatman filter paper. The organic solvent (ethanol) was evaporated with a rotary evaporator at room temperature to get the dried crude extract (yield value of 2.064%). Then the dried crude extract was stored in the refrigerator at 4 °C.

Reagents and chemicals
Ascorbic acid and acetic acid were purchased from Merck, Germany, while tween-80 was purchased from Loba Chemie Pvt. Ltd., India. Solvents and all the other reagents and chemicals were of analytical grade. The diclofenac sodium was collected from Aristopharma Ltd., Bangladesh, while the standard antibiotic discs (Ciprofloxacin 5 μg/disc) were purchased from Oxoid Ltd., UK, and sterile blank discs were also purchased from BBL, Cocksville, USA. Streptokinase (Durakinase) powder for reconstitution; 1,500,000 units/vial used in the clot lysis test was purchased from Dong Kook Pharmaceutical Co. Ltd., South Korea.

Experimental animals
Adult male albino mice (21–34 g), purchased from the animal resource branch of Jahangirnagar University, Dhaka, were used for the study. The animals were housed under standard environmental conditions (temperature: 25 ± 2 °C, humidity: 50 ± 5%, and 12 h light/dark cycles) in sanitized polypropylene cages containing sterile paddy husk as bedding. Animals were freely accessing standard pellets as a basal diet and water ad libitum. All the animals were acclimatized for 2 weeks before starting the study. The animals were randomized into experimental and control groups and the food was withdrawn 12 h before the experimental hours.

Phytochemical investigation
Preliminary phytochemical group tests were done to investigate plant secondary metabolites such as alkaloids, glycosides, flavonoids, steroids, terpenoids, sapo-nins, tannins, gums, and reducing sugars in the extracts by the methods of Harborne [19] and Trease and Evans [55].

Anti-inflammatory activity test (egg albumin model)
To determine the anti-inflammatory effect of the crude extract, the egg albumin model was used [56] with a slight modification. In brief, the inhibition of protein denaturation indicates the anti-inflammatory effect of a substance (e.g., extract, drug). In this study, B. pilosa crude extract at 5–320 μg/mL was tested by taking distilled water and acetyl salicylic acid as negative and positive controls, respectively. The crude extract was prepared in a similar manner as mentioned in the previous test (clot lysis assay). The 5 mL reaction mixture consisted of egg albumin (0.5 mL, from fresh hen’s egg), phosphate buffered saline (3.5 mL) (pH 6.4) and varying concentrations of test sample (1 mL). Then the mixtures were incubated at (37 ± 2 °C) in a bio-oxygen demand (BOD) incubator for 15 min and then heated at 70 °C for 5 min. After cooling, the absorbance was measured at 660 nm (UV/VIS Spectrophotometer, Shimadzu, Japan) by using the vehicle as a blank. A similar volume of distilled water and acetyl salicylic acid (100 μg/mL) were used as negative and positive controls, respectively. The percentage of inhibition of protein denaturation was calculated by using the following formula:

\[
\% \text{Inhibition} = \left(\frac{\text{Absorption}_{\text{Control}} - \text{Absorption}_{\text{Test}}}{\text{Absorption}_{\text{Control}}}\right) \times 100
\]

Thrombolytic activity test
The venous blood drawn from healthy volunteers (n = 8) was distributed into the negative control (distilled water); standard (streptokinase); and 6 different concentrations (5 to 320 μg/mL) of the crude extract marked alpin tubes. For this purpose, the required amount of crude extract was dissolved in a small amount of ethanol, which was then reconstituted in distilled water qsto. Each tube was pre-weighed (W1) and contained 0.5 mL of blood. The tubes were then incubated at 37 °C for 45 min. After clot formation, serum was completely removed carefully (without disturbing the clot) and again weighed (W2) to determine the clot weight (W2-W1). After the addition of test sample/controls (100 μL/tube), the tubes were then re-incubated at 37 °C for 90 min. Then the lysed fluid was removed carefully and the tubes were weighed (W3) to observe the difference in weight after clot disruption (W3-W2). The difference obtained in weight taken before and after clot lysis was expressed as a percentage of clot lysis [45].

Anti-nociceptive activity test (acetic acid induced writhing model)
This study was performed according to the method described by Koster et al. [27] and Ezeja et al. [15]. For this purpose, the experimental animals (Swiss mice) were randomly divided into 4 groups, namely negative control (Vehicle), positive control (Diclofenac sodium, 25 mg/kg), and 2 test groups (250 and 500 mg/kg). The control group received 0.5% tween-80 dissolved in a 0.9% NaCl solution. All the treatments were given via oral gavage at a dose of 10 mL/kg body weight. After 30 min of
treatments, each mouse was treated with 0.7% acetic acid solution intra-peritoneally and the number of writhings provided by each animal was counted for 10 min.

Statistical analysis
The results were presented as mean ± SEM. A student’s t-test was used to determine if there was a significant difference between the experimental and control groups. The test results were statistically significant when p < 0.05 at 95% confidence intervals. Half maximal effective concentrations (EC50s) were calculated by extrapolating the test concentration vs. responses (non-linear method) using GraphPad Prism software (version: 6.0, San Diego, California, USA. copyright© 1994–1999), considering p < 0.05 at a 95% confidence interval.

Results
Phytochemical studies
Table 1 suggests that the crude ethanol extract of B. pilosa possesses alkaloids, glycosides, flavonoids, terpenoids, steroids, tannins, saponins, and reducing sugars. More intensity was seen in the case of terpenoids, followed by alkaloids, glycosides, tannins, saponins, and reducing sugars. Poor intensity was seen in the case of the flavonoids and steroids tests, while no intensity was observed for the gum test.

Anti-inflammatory activity
B. pilosa extract concentration-dependently inhibited protein denaturation in the test tubes. The extract exhibited the highest (67.53 ± 0.33%) inhibition of albumin denaturation at 320 μg/mL concentration. ASA at 5 to 100 μg/mL showed total inhibition of protein denaturation by 21.23 ± 0.23 to 72.78 ± 0.07%, while the extract of B. pilosa at 5 to 320 μg/mL showed total inhibition of protein denaturation from 12.73 ± 0.17 to 67.53 ± 0.33%. Thus, the standard ASA at 100 μg/mL exhibited a better inhibitory effect than the test sample. The half maximal effective concentrations (EC50s) calculated for the extract and ASA were 125.30 ± 0.11 and 34.08 ± 0.23 μg/mL, respectively (Table 2).

Thrombolytic activity
The extract showed significant (p < 0.05) clot lysis capacity in a concentration-dependent manner. The extract of B. pilosa at 10 to 320 μg/mL showed clot lysis capacity of 8.83 ± 0.20 to 51.32 ± 0.73%. The highest clot lysis of the extract was 51.32 ± 0.73% at 320 μg/mL. The standard, SK, and NC produced clot lysis of 81.08 ± 0.01 and 2.57 ± 0.18%, respectively. The EC50 calculated for the test extract was 217.10 ± 0.08 μg/mL [CI: 132.30–356.10 μg/mL; R2: 0.90] (Table 3).

Acetic acid induced writhing test
The B. pilosa ethanol extract dose-dependently modulated the anti-nociceptive parameters in experimental animals. The extract at both doses (250 and 500 mg/kg, p.o.) and the standard drug, diclofenac sodium (25 mg/kg, p.o.) significantly (p < 0.05) modulated the anti-nociceptive parameters in the test animals in comparison to the NC group. B. pilosa extract at 500 mg/kg showed writhing and %protection of 16.20 ± 1.16 and 54.75%, respectively, while the standard (25 mg/kg) showed writhing and %protection of 7.80 ± 1.32 and 78.21%, respectively (Table 4).

Discussion
Solvent extraction is one of the safest methods for the extraction of a wide variety of medicinal plants. High proof alcohols (190 and 210) are used for extraction applications. Ethanol is the emerging and more popular solvent, as it is safe for infusing edibles. It is compatible with any type of container-easily recoverable and it provides consistent results during the evaluation of the crude extract. In this study, we also prepared an extraction with this popularly used solvent.

Inflammation is a major biological process that regulates the interaction between organisms and the environment [8]. Chronic and low-grade inflammation may cause cellular aging and many diseases, including arthritis, allergies, atherosclerosis, and cancer [16]. Tissue protein denaturation may produce auto-antigens in certain arthritic diseases. Therefore, it is a marker for inflammation and arthritic diseases [1]. Protein denaturation results in inflammation through loss of tertiary and secondary structures of proteins from the effects of heat, strong acid/base, inorganic salt or organic solvents [26]. Anti-inflammatory drugs can be used to inhibit protein denaturation [43]. In this study, B. pilosa extract showed significant anti-inflammatory activity by inhibiting the heat-induced lysis of egg albumin. Phytochemicals such as alkaloids and flavonoids present in the plant are responsible for giving anti-inflammatory effects [3].

Table 1 Secondary metabolites in B. pilosa ethanol extract

| Phytochemical groups | Test results |
|----------------------|-------------|
| Alkaloids            | ++          |
| Glycosides           | ++          |
| Flavonoids           | +           |
| Terpenoids           | +++         |
| Steroids             | +           |
| Tannins              | ++          |
| Saponins             | ++          |
| Gums                 | –           |
| Reducing sugars      | ++          |

Intensity: (−) → No, (+) → Low, (+++) → Moderate, (++++) → Strong
Moreover, terpenoids [18], glycosides and other plant-derived secondary metabolites are the potential sources of anti-inflammatory agents [40]. In the present study, we also found diverse secondary metabolites in *B. pilosa* extract, suggesting a strong foundation for further studies aiming to isolate anti-inflammatory lead compounds from this medicinal herb. In a recent study, the methanol extract has been reported to have a concentration-dependent (62.5 to 500 μg/mL) membrane stabilizing activity [22]. The highest percentage inhibitory observed in the case of ethanol extract in this study is comparable to the highest concentration (320 μg/mL) of methanol extract.

According to the World Health Organization (WHO), approximately 65% of the world's population uses traditional medicine for medical care [40]. Plants are the reservoirs of potential secondary metabolites (major sources of drugs), that can be used to treat diverse pathological states in humans [53]. Several anti-inflammatory medicinal plant species, such as *Myracrodruon urundeuva* Allemão, *Schinus terebinthifolius* Raddi, *Spondias momin* L., *Spondias purpurea* L., *Spondias tuberosa* Arruda, *Euphorbiaceae acalypha* hispida Burm. f., *Acalypha indica* L. and *Phyllanthus niruri* L. [4, 9, 38, 47, 54, 61]. The authors reported that it may be due to the presence of phenols, triterpenes, flavonoids, saponins, and tannins in their extracts. The current study also reports that *B. pilosa* ethanol extract also possesses terpenoids, saponins, and tannins. Thus, the anti-inflammatory effects of *B. pilosa* might be due to its above-mentioned secondary metabolites.

### Table 2 Total inhibition of protein denaturation by the *B. pilosa* ethanol extract and the controls

| Treatments            | Egg albumin test |       |
|-----------------------|------------------|-------|
|                       | TIPD             | EC_{50} (CI; R²) |
| NC                    | –                | –     |
| ASA (μg/mL)           | 5                | 21.23 ± 0.23 | 34.08 ± 0.23 μg/mL (19.34–60.04 μg/mL; 0.87) |
| 10                    | 27.31 ± 0.34     |       |
| 20                    | 32.21 ± 0.17     |       |
| 40                    | 52.43 ± 0.07     |       |
| 80                    | 57.23 ± 0.43     |       |
| 100                   | 72.78 ± 0.07     |       |
| *B. pilosa* (μg/mL)   | 5                | 12.73 ± 0.17 | 125.30 ± 0.11 μg/mL (69.30–226.60 μg/mL; 0.89) |
| 10                    | 24.68 ± 0.43     |       |
| 20                    | 36.49 ± 0.36     |       |
| 40                    | 42.86 ± 0.27     |       |
| 80                    | 48.05 ± 0.23     |       |
| 160                   | 59.35 ± 0.17     |       |
| 320                   | 67.53 ± 0.33     |       |

Values are Mean ± SEM (n = 5)

NC negative control, ASA acetyl salicylic acid, TIPD total inhibition of protein denaturation, EC_{50} half-maximal effective concentration, CI confidence of intervals, R² co-efficient of determination

**Table 3 Clot lysis capacity of *B. pilosa* ethanol extract and the controls**

| Treatments            | % of clot lysis | EC_{50} (CI; R²) |
|-----------------------|-----------------|-----------------|
| NC                    | 2.57 ± 0.18     | –               |
| SK (100 μl (30,000 I.U.)) | 81.08 ± 0.01* | –               |
| *B. pilosa* (μg/ml)   |                 |                 |
| 10                    | 8.83 ± 0.20*    | 217.10 ± 0.08 μg/mL (132.30–356.10 μg/mL; 0.90) |
| 20                    | 10.99 ± 0.44*   |                 |
| 40                    | 13.42 ± 0.39*   |                 |
| 80                    | 21.15 ± 1.15*   |                 |
| 160                   | 36.31 ± 0.03*   |                 |
| 320                   | 51.32 ± 0.73*   |                 |

Values are mean ± SEM (n = 3); t-student’s test at 95% confidence intervals

EC_{50} half-maximal effective concentration, CI confidence of intervals, R² co-efficient of determination, SK streptokinase

* p < 0.05 when compared to the NC (negative control) group
Atherothrombosis, characterized by the formation of one or more atheromatous plaques inside the blood vessels, is one of the major consequences of morbidity and mortality worldwide [33, 42]. The fibrinolytic agents are used to lyse the clotted blood inside the vessels [2]. Alteplase, anistreplase, streptokinase, urokinase, and tissue plasminogen (TPA) are also used to treat thrombosis [25]. Among them, streptokinase and urokinase are widely used due to their low cost [10, 39]. Cumulative reports suggest that herbal products have promising anti-thrombotic effects [21, 30]. Plant-derived secondary metabolites such as polyphenols [7], alkaloids [41], and glycosides [63] are evident to possess anti-atherothrombosis activity. Streptokinase is an extracellular fibrinolytic enzyme secreted by the beta-hemolytic Streptococci bacteria [31]. It works through binding to both free and fibrin bound plasminogen to form a complex, and subsequently converts the other free plasminogen into an activated protease plasmin, thus dissolving the clotted blood in the blood vessels [35]. The most frequent use of this drug is in the treatment of acute myocardial infarction, deep vein thrombosis, and pulmonary embolism [14]. Our study demonstrates that B. pilosa extract has significant concentration-dependent clot lysis capacity. It is to be mentioned that obesity-driven chronic inflammation along with an impaired fibrinolysis event appear to be one of the major effector mechanisms of thrombosis in obesity [6]. One study reports that the methanol extract of B. pilosa is evident to exert an anti-obesity effect on Swiss albino mice [62]. Therefore, anti-obesity and clot lysis capacity might be related to each other. Sedighi et al. [52] report that medicinal plants are a hope for treating atherosclerosis through reducing cholesterolemia, free radicals, inflammation, vascular resistance, and important enzymes. Thus, the medicinal plant alone or in combination with hypcholesterolemic drugs, can be a useful tool for the treatment of patients with hyperlipidemia and its complications. In a study, zinc chelation was found to promote streptokinase-induced thrombolysis in vitro [58].

Acetic acid causes the release of several endogenous substances (e.g., serotonin, bradykinins, histamine, prostaglandins (PGs), substance P) that are liable to cause pain by accelerating nerve endings. Locally sensitized peritoneal receptors are responsible for the activation of abdominal constrictions response [48]. This model has also been connected with prostanoids that increase the levels of PG-\(\text{E}_2\) and \(-\text{E}_{2\alpha}\) in peritoneal fluids, as well as lipoygenase products [60]. In this study, B. pilosa extract was found to exhibit a significant anti-nociceptive effect on test animals. The inhibition of synthesis and release of PGs and other endogenous substances may be the most probable pathway for peripherally acting analgesics. Jyoti et al. [22] have reported a dose-dependent analgesic effect in the formalin-induced paw licking mouse model. Our study also demonstrates a dose-dependent anti-nociceptive effect in an acetic acid induced writhing mouse model. In both cases, the activity observed is comparable. It seems our reports agree with the findings of Jyoti et al. [22].

The analgesic profiles of many plant species are widely used in folk medicine, for example, Hyptis pectinata Poit. (Lamiaceae) [5], Erythrina velutina Willd. (Fabaceae) [11], and Hyptis fruticosal Salzm. ex Benth. (Lamiaceae) [37]. These plants are evident to contain terpenoids. Thus, terpenoids may be important tools for pain management in animals. In general, pain is an unpleasant sensation that is associated with real or potential tissue damage. The experience of pain is generated in the early stages by direct activation of the sensory nerve fibers. The discomfort in the late phase, on the other hand, is related to inflammatory mediators. Current anti-inflammatory and pain medications are helpful, but they have serious side effects such as ulcers, anemia, osteoporosis, and endocrine disruption. Consequently, there has been a greater emphasis on the investigation of the analgesic potential of phytoconstituents, or traditional medicinal plants, because they frequently exhibit adequate biological activity with fewer side effects when compared to manufactured medicines [24]. The current study demonstrates that 500 mg/kg (p.o.) of B. pilosa ethanol extract showed a significant analgesic effect on the experimental animal. Thus, the potent anti-inflammatory and analgesic effects of its highest concentration/dose may co-relate with each other.

Our study reports that B. pilosa contains many important secondary metabolites. The practice of treating diseases by using medicinal plants and their derivatives dates back to ancient civilizations. Plant secondary metabolites are good sources for treating diverse ailments (Hu et al., 20,020). Among the secondary metabolites, terpenoids, phenolics, flavonoids, alkaloids and glycosides are known as important ingredients in nutraceuticals as well as modern medicines [49, 59]. Generally, most secondary metabolites have potent antioxidant properties. Therefore, they have protective capacity [44].

### Table 4 Effects of B. pilosa ethanol extract and controls on acetic acid induced writhing mice

| Treatments per oral          | Writhing     | % protection |
|-----------------------------|--------------|--------------|
| NC (10 ml/kg)               | 35.80 ± 1.07 | 0            |
| PC (25 mg/kg)               | 7.80 ± 1.32* | 78.21        |
| Extract (mg/kg)             | 250          | 24.60 ± 1.21* | 31.28        |
|                             | 500          | 16.20 ± 1.16* | 54.75        |

Values are mean ± SEM (n = 5) and percentage; t - student’s test at 95% confidence intervals.
NC negative control (Vehicle), PC positive control (Diclofenac sodium)
\(p < 0.05\) when compared to the NC (negative control) group
These metabolites are evident to impart a wide range of therapeutic activities; they can directly interact with receptors, cell membranes, and genetic materials (e.g., DNA, RNA) [57].

Risk/benefit evaluation of a herbal product is crucial. For this, pre-clinical and non-clinical studies play important roles in the study of the safety potential of a wide variety of crude drugs of natural origins. Experimental animals such as mice and rats are frequently used for this purpose [36]. According to Jyoti et al. [22], the methanol extract up to 3000 mg/kg (p.o.) of this herb did not show acute toxicity in Swiss mice. It seems the extract is well-tolerated in Swiss mice.

Conclusion
Our study suggests that B. pilosa possesses many important secondary metabolites such as alkaloids, flavonoids, glycosides, terpenoids, tannins, saponins, and reducing sugars. B. pilosa has shown significant thrombolytic, anti-inflammatory, and anti-nociceptive activities on the test systems applied in our study. B. pilosa might be a hopeful alternative medicinal plant to fight against various diseases, including atherothrombosis, inflammation and inflammatory diseases. However, the molecular interactions between the phytochemicals of this herb and the observed biological activities in this study are yet to be determined. Therefore, further studies are necessary to isolate the lead compounds from the hopeful medicinal herb and evaluate the possible mechanism(s) for each pharmacological effect by adopting suitable test models.

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Authors’ contributions
L. Sikder- laboratory studies, data collection, preliminary drafting; M. R. Khan- laboratory studies, data collection; S. Z. Smrity- laboratory studies, data collection; M. T. Islam- data manipulation, data analysis, final drafting; S. A. Khan- work design, data manipulation, data analysis, final drafting. The author(s) read and approved the final manuscript.

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Declarations
Ethics approval and consent to participate
This project was approved by the Department of Pharmacy (Approval No. 12–17/95/bsmrstu/2017), BSMRSTU, Gopalganj-6100, Bangladesh.

Consent for publication
The authors declare their consent for publication.

Competing interests
The authors declare no conflict of interest.

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