Research Article

Phytochemical and Pharmacological Profiling of *Heritiera fomes* Buch. Ham. Deciphered Thrombolytic, Antiarthritic, Anthelmintic, and Insecticidal Potentialities via In Vitro Approach

Farhana Alam Ripa, Md. Jamal Hossain, Mst. Luthfun Nesa, Miss Sharmin Zahan, Saikat Mitra, Mohammad A. Rashid, Arpita Roy, Saad Alghamdi, Mazen Almehmadi, and Osama Abdulaziz

1Department of Pharmacy, Brac University, 41-Pacific Tower, Mohakhali, Dhaka 1212, Bangladesh
2Department of Pharmacy, State University of Bangladesh, 77 Satmasjid Road, Dhanmondi, Dhaka 1205, Bangladesh
3Department of Pharmacy, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh
4Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh
5Department of Biotechnology, School of Engineering & Technology, Sharda University, Greater Noida, India
6Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia
7Clinical Laboratory Sciences Department, College of Applied Medical Sciences, Taif University, Taif, Saudi Arabia

Correspondence should be addressed to Md. Jamal Hossain; jamal.du.p48@gmail.com and Arpita Roy; arbt2014@gmail.com

Received 30 May 2022; Accepted 24 June 2022; Published 18 July 2022

Academic Editor: Shuli Yang

Copyright © 2022 Farhana Alam Ripa et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Medicinal plants have been crucial in treating various chronic ailments since ancient times. The objective of this study was to evaluate in vitro pharmacological properties of petroleum ether, chloroform, and ethyl acetate soluble fractions of ethanolic extract (leaf, bark, and root) of *Heritiera fomes* Buch. Ham., including the phytochemical screening of the plant. Thrombolytic and antiarthritic properties were assessed through the clot lysis and protein denaturation experimental method, correspondingly. Anthelmintic and insecticidal activities were studied against *Pheretima posthuma* and *Tribolium castaneum*, respectively. The phytochemical analysis exhibited numerous active phytochemicals in different solvent fractions. In thrombolytic investigation, among all crude extracts, ethanolic leaf extract showed the highest 33.12 ± 7.52% clot lysis as compared to standard streptokinase (67.77 ± 9.78%). In antiarthritic assay, all the tested samples exhibited noteworthy protein denaturation in dose-dependent manner (100–500 μg/mL), whereas the utmost percentage inhibition was noticed for chloroform extract of roots (63.28 ± 5.96% at 500 μg/mL). All crude extracts exhibited a significant anthelmintic activity in different concentrations (25–75 mg/mL) and revealed paralysis and death of earthworms in comparison with albendazole; ethanolic extract of the bark was found to be more potent at the highest dose. For the insecticidal test, ethanolic extract of the leaf showed the utmost mortality rate (73%). The outcomes of the investigation confirmed the potential thrombolytic, antiarthritic, anthelmintic, and insecticidal activities of the different extracts of *H. fomes*, and hence, advanced studies on the isolation and identification of active phytocompounds are highly needed for new drug development.

1. Introduction

Nature is often a brilliant sign to point out the distinguished marvels of existence. Natural merchandise from plants, animals, and minerals area unit the premise for treating numerous human diseases [1, 2]. Herbs, particularly medicative herbs, have perpetually acted as an associate overall indicator of scheme health [3]. According to the World Health Organization (WHO), 65–85% of the world’s population still depends on plants as a resource for primary healthcare [4–6]. Plant-derived remedies function as a model to develop more efficient and less noxious medicines.
The healing properties of medicinal plants are possibly owing to the existence of miscellaneous secondary metabolites like alkaloids, glycosides, flavonoids, phenols, saponins, and sterols [7–9]. There is an escalating attention in correlating the phytochemicals of a medicinal plant with its medicinal property [10, 11].

Bangladesh possesses a rich flora and expanded genetic resources of medicinal plants [12–14]. Reports proved that in vitro screening approaches could offer the required preliminary remarks necessary to choose crude plant extracts with potentially valuable properties for advanced chemical and pharmacological investigations [15, 16]. This information exhilarated us to find out new hope for medical science from Heritiera fomes Buch. Ham. plant. It is an important moderate size mangrove tree growing copiously in the Sundarbans [17, 18]. The earlier research showed that H. fomes contains alkaloids, glycosides, flavonoids, saponins, carbohydrates, phenols, gums, and sterols, which were confirmed during their phytochemical investigations of different plant parts extracts [17, 19]. The plant is used in different types of gastrointestinal and skin disorders, diabetes, goiter, and cardiovascular diseases. It also possesses wound healing, antioxidant, antinociceptive, antimicrobial, anticancer, and insect-repelling properties [17, 19–21].

Although few studies were conducted earlier on the ethnomedicinal properties of H. fomes, no record has been found on its in vitro thrombolytic, antiarthritic, anthelmintic, and insecticidal activities. Therefore, the study was conducted to assess the in vitro thrombolytic, antiarthritic, anthelmintic, and insecticidal properties of ethanolic extract and its three different partitions (ethyl acetate, petroleum ether, and chloroform) of leaf, bark, and root of H. fomes along with the phytochemical screening with a view to exploring its possible applications in pharmaceutics.

2. Materials and Methods

2.1. Plant Materials. Fresh leaves, barks, and roots of H. fomes were collected from the Sundarbans, Bagerhat district, Bangladesh, in December 2020. The plant was identified and authenticated by a taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No: 50664), and was preserved in our laboratory for future reference. The collected plant parts were washed cautiously with running tap water to remove dirt and then rinsed with distilled water. The leaves, barks, and roots were separated, cut into small pieces, shade dried for two weeks, and pulverized into a coarse powder with a laboratory electric blender. The powdered plant materials were stored separately in airtight containers in a cool, dark, and dry place for further use.

2.2. Chemicals and Reagents. All the reagents were analytical reagent grades procured from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). 5 ml phosphate buffered saline was added to the commercially available lyophilized streptokinase (15,000,000 IU) vial (S-kinase, Popular Pharmaceuticals Ltd., Bangladesh) and mixed accurately. The concentration of the streptokinase was accustomed to being 30,000 IU and used as the reference standard for thrombolytic activity. Diclofenac sodium and albendazole were collected from Square Pharmaceutical Ltd., Bangladesh.

2.3. Extraction Procedure. The powered plant materials of leaf, bark, and root (500 gm) were taken into three different clean glass containers and soaked in 2 L ethanol for a week with random shaking and stirring. The mixture was then individually filtered by Whatman filter paper (number 1) into three different clean beakers. The filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure. Around 5 g of the concentrated extracts (leaf, bark, and root) of H. fomes was subjected to solvent-solvent partitioning following the modified Kupchan partitioning procedure [22] into petroleum ether, chloroform, and ethyl acetate soluble portions and were individually assessed for phytochemical and in vitro biological activities. The ethanol, petroleum ether, chloroform, and ethyl acetate extracts of the leaf were tagged as LE, LPE, LC, and LEA, respectively. Bark extracts were labeled as BE (ethanolic), BPE (petroleum ether), BC (chloroform), and BEA (ethyl acetate); whereas, root extracts were marked as RE, RPE, RC, and REA for ethanol, petroleum ether, chloroform, and ethyl acetate solvents, correspondingly.

2.4. Phytochemical Analysis. The freshly prepared crude extracts were qualitatively tested for the presence of active bioactive phytochemicals like carbohydrates, alkaloids, glycosides, flavonoids, saponins, sterols, tannins, fixed oil, resins, and phenols by using standard procedures [23].

2.5. Thrombolytic Activity. In vitro thrombolytic potentiality of the experimented extracts was evaluated by applying the method described by Tabassum et al. [24]. Venous blood samples (4 mL) were collected from healthy human volunteers (n = 10) with no hematological disorders or with any history of taking anticoagulant therapy. Then, the collected blood sample was transferred to several preweighed sterile microcentrifuged tubes (1 mL/tube). Informed written consent was taken from each volunteered blood donor. One mL of blood was transferred to each preweighed sterile Eppendorf tube and incubated at 37°C for 45 minutes. The serum was completely eradicated after clot formation. Each tube having a clot was further weighted to measure the clot weight (clot weight = weight of clot containing tube – weight of tube alone). Then, 100 μL of different crude extracts was added to each of these tubes separately. Here, we used streptokinase and distilled water as positive control and negative control, respectively. Then, 100 μL of streptokinase and 100 μL of distilled water were individually added to the control marked Eppendorf tubes. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the released fluid was discarded, and tubes were again weighted to see the difference in weight after clot disruption. The difference obtained in weight taken before...
and after clot lysis was represented as a percentage of clot lysis. The experiment was done three times with different blood samples of volunteers. We have used the following formula to calculate the % of clot lysis [24]:

\[
% \text{ clot lysis} = \left( \frac{\text{Weight of the clot lysis}}{\text{Weight of clot before lysis}} \right) \times 100.
\]

2.6. Antiarthritic Activity. To assess antiarthritic activity, we followed the method described by Naz et al. [25]. At first, 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of different experimented extracts at various concentrations (100–500 μg/mL) were mixed together. Then, all the samples were incubated at 37°C for 30 minutes and further heated at 57°C for 3 minutes to induce denaturation of protein. Later, 2.5 ml phosphate buffer (pH 6.3) was added to each tube after cooling them to room temperature and absorbance of turbidity was measured at 660 nm. Instead of the extract, we used 0.05 ml distilled water solution as control. In this experiment, diclofenac sodium was used as standard at the same concentrations as the crude extracts and treated similarly. The result was calculated by using the following formula:

\[
\% \text{ inhibition} = \frac{V_c - V_t}{V_t} \times 100,
\]

where \( V_t \) is the absorbance of test samples; \( V_c \) is the absorbance of control.

2.7. Anthelmintic Activity. The anthelmintic activity of \( H. fomes \) extracts was assayed by the modified Gopalakrishnan et al.’ [26] method. Here, we used Bangladeshi earthworms (\( Pheretima posthuma \)) because of its anatomical and physiological semblance with the human intestinal roundworm parasite [27]. After collection from moist soil, all worms were washed with normal saline water to eradicate all faecal matter and accustomed with laboratory environment before experimentation. In this experiment, we have used freshly prepared standard and test solutions. Test samples of the experimented extracts were prepared at the concentrations of 20–75 mg/ml in Tween 20 (1%) solution diluted with normal saline. Nearly equal sized earthworms were divided into seven groups (consisting of six worms in each) and were released into 30 ml of experimental formulation. The first group received Tween 20 along with normal saline and was taken as control; group two was treated with reference drug albendazole at a concentration of 20 mg/ml and considered as standard. Groups 4–7 were treated with different solvent extracts of \( H. fomes \) in various concentrations (20 mg/ml, 25 mg/ml, 50 mg/ml, and 75 mg/ml). Time of paralysis and time of death of the experimented worms were noted. Time for paralysis was considered when any kind of movement could not be perceived excluding when the worms were shaken robustly. Time of death was concluded and documented after discerning that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C) followed with fading away of their body colors.

2.8. Screening of Insecticidal Activity. Insecticidal activity of the experimented samples was assayed against \( Tribolium castaneum \). These insects were collected from the stock cultures of the Crop Protection and Toxicology Laboratory, Sher-e-Bangla Agricultural University, Bangladesh. To perform the experiment, the test samples were prepared into six different concentrations (2.5 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ml, 40 mg/ml, and 50 mg/ml) by dissolving the extracts into respective solvents. Then, the solutions were poured separately on the lower part of individual 60 mm Petri dishes and stand them for a while in open air to evaporate solvents. Then, six insects were placed in each of the treated Petri dishes including the control and kept them all in a secured place at room temperature. Three replicates were set up for the treated and control solutions. The mortality of the insects was checked initially after 30 minutes from the beginning and then later 1, 2, 4, 8, 12, and 48 h of exposure and the data were taken. Here, we used a simple microscope to trace the natural movement of its organs. Often, we used a warm needle closer to the bodies (lack of movement) to confirm death. The mortality record of the adult \( T. castaneum \) was calculated by the following formula [28]:

\[
\text{Pr} = \frac{\text{Po} - \text{Pc}}{100 - \text{Pc}} \times 100.
\]

Pr is the percentage (%) of corrected mortality; Po is the observed mortality; Pc is the control mortality, sometimes called natural mortality.

2.9. Statistical Analysis. In this study, all the in vitro experiments were performed in three replicates. Statistical analysis was completed by using SPSS software version 19.0. The results were articulated as mean ± standard error of mean (SEM), and Student’s t-test and one way ANOVA followed by Dunnett’s post hoc multiple comparison test were used to determine the values of \( P \). The outcomes beneath \( P < 0.05 \) were considered as significant.

3. Results

3.1. Phytochemical Screening. The phytochemical screening of the experimented extracts of \( H. fomes \) revealed the presence of alkaloid, carbohydrate, tannins, flavonoid, saponin, glycoside, steroid, phenol, and resin (Table 1).

3.2. Thrombolytic Activity. When we added 100 μl streptokinase (30,000 IU) to the clots along with 90 minutes incubation at 37°C, it exhibited a significant (67.77 ± 9.78%; Table 2) clot lysis; whereas, clots treated with 100 μl sterile distilled water (negative control) revealed only insignificant clot lysis (10.44 ± 1.72%; Table 2). Here, we found that all the experimented solvent extracts of \( H. fomes \) possess thrombolytic activity. Among them, ethanolic extract of leaf
Table 1: Screening of bioactive phytocompounds in different extracts of *H. fomes*.

| Phytocompounds | Leaf | Root | Bark |
|----------------|------|------|------|
| Carbohydrate   | LE   | LEA  | LC   | LPE  | RE   | REA  | RC   | RPE  | BE   | BEA  | BC   | BPE  |
| Glycoside      | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Tannin         | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Alkaloid       | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Saponin        | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Resin          | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Phenol         | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Flavonoid      | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Steroid        | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Fixed oil      | −    | −    | −    | −    | −    | −    | −    | −    | −    | −    | −    | −    |

Values are expressed as mean ± S.E.M. (n = 3). Data were analyzed by ANOVA followed by Student’s *t*-test, *p* < 0.01 and ∗∗ *p* < 0.001.

revealed the highest percentage of clot analysis (33.12 ± 7.52%; Table 2). In contrast, ethyl acetate extract of bark showed the lowest level of clot analysis (20.97 ± 6.97%; Table 2). The detailed statistical representation of the effective clot lysis percentage by negative control (sterile distilled water), positive control (streptokinase), and different extracts is given in supplementary Table S1.

3.5. Screening of Insecticidal Activity. The insecticidal activity of *H. fomes* extracts is shown in Figures 1 and 2. Besides, the detailed statistical data of the experiment for assessing the insecticidal activity are found in supplementary Table S4. We noticed that their insecticidal potency increased in dose-dependent manner. The highest mortality rate was observed for ethanolic extract of leaf (73%).

4. Discussion

The interaction between humans and plants has long been described as one of the factors influencing human civilization, especially in the field of medicine [29, 30]. Still, 80% of people in developing countries prefer herbal formulations [31–33]. A phytopharmacological study has unwrapped a unique zone to discover plant-based medicines that are effective for the cure of different diseases and has been approved by the Food and Drug Administration [34]. *H. fomes* is a major mangrove species. Earlier studies reported the existence of alkaloids, tannins, polyphenols, steroids, saponins, glycosides, flavonoids, reducing sugars, gums, and carotenoids in different parts of *H. fomes* [17, 19], and these have been confirmed in parts by our own findings. Despite having huge potential, inadequate reports are available about its biological activities [17, 19–21]. These motivated us for further assay of different pharmacological activities of various parts of *H. fomes*. In this study, we have checked the in vitro thrombolytic, antiarthritic, anthelmintic, and insecticidal properties of ethanolic and its different solvent fractions of the experimented plant parts and
Table 3: In vitro antiarthritic activity of *H. fomes* extracts in different concentrations.

| Sample   | 100 µg/mL   | 200 µg/mL   | 300 µg/mL   | 400 µg/mL   | 500 µg/mL   |
|----------|-------------|-------------|-------------|-------------|-------------|
|          |            |            |            |            |            |
| Diclofenac sodium | 61.63 ± 1.44 | 71.41 ± 1.49 | 78.13 ± 1.47 | 81.09 ± 3.08 | 87.45 ± 4.05 |
| LPE      | 45.35 ± 2.8* | 46.78 ± 4.1* | 48.62 ± 5.2* | 49.69 ± 7.09 | 60.59 ± 6.66* |
| LEA      | 42.82 ± 4.4* | 47.45 ± 4.5* | 49.99 ± 5.61* | 50.65 ± 7.76* | 59.53 ± 8.66* |
| LC       | 36.73 ± 6.0* | 39.89 ± 6.4* | 45.98 ± 6.15* | 47.61 ± 5.06* | 48.93 ± 8.42* |
| LE       | 41.19 ± 6.6* | 46.11 ± 5.0* | 48.15 ± 5.39* | 55.53 ± 6.02* | 62.08 ± 6.31* |
| RPE      | 39.82 ± 5.5* | 43.97 ± 5.6* | 46.53 ± 5.53* | 54.04 ± 6.16* | 57.35 ± 8.73* |
| REA      | 40.25 ± 5.1* | 43.97 ± 5.6* | 48.29 ± 7.6*  | 52.34 ± 6.72* | 55.92 ± 8.31* |
| BC       | 41.36 ± 4.6* | 50.53 ± 4.0* | 52.05 ± 6.58* | 53.75 ± 6.19* | 63.28 ± 5.96* |
| RE       | 36.12 ± 5.7* | 38.06 ± 7.2* | 46.61 ± 6.51* | 49.02 ± 7.28* | 56.73 ± 7.75* |
| BPE      | 38.85 ± 5.7* | 39.00 ± 7.3* | 41.84 ± 7.5*  | 47.96 ± 6.94* | 55.33 ± 7.82* |
| BEA      | 41.48 ± 5.3* | 45.69 ± 6.3* | 49.03 ± 6.31* | 56.21 ± 6.51* | 61.49 ± 6.73* |
| BC       | 38.14 ± 6.0* | 42.87 ± 5.3* | 48.25 ± 6.73 | 50.84 ± 8.27* | 60.14 ± 7.54* |
| BE       | 40.96 ± 5.18* | 44.11 ± 5.45 | 52.03 ± 6.5*  | 53.78 ± 7.01* | 55.88 ± 7.72* |

Values are expressed as mean ± S.E.M (n = 3). Data were analyzed by ANOVA followed by Dunnett’s *t*-test, *p < 0.01.

Table 4: Anthelmintic activity of *H. fomes* extracts in different concentrations.

| Treatment | Conc. used (mg/ml) | Time taken for paralysis (min) | Time taken for death (min) |
|-----------|--------------------|-------------------------------|-----------------------------|
| Control Standard | 20 | 37.67 ± 1.53 | 45.33 ± 2.52 |
| Standard | 25 | 50.67 ± 5.13* | 69.00 ± 7.55* |
| BE       | 50 | 44.00 ± 2.00* | 54.33 ± 2.52* |
| 75       | 39.67 ± 2.52 | 49.67 ± 3.51 |
| LE       | 25 | 59.67 ± 8.08* | 67.00 ± 7.09* |
| 50       | 48.33 ± 6.66* | 57.33 ± 3.05* |
| 75       | 46.00 ± 5.29 | 52.67 ± 4.61 |
| RE       | 25 | 52.33 ± 9.45 | 69.67 ± 8.02* |
| 50       | 44.00 ± 7.81 | 54.00 ± 7.81 |
| 75       | 42.00 ± 2.64 | 52.33 ± 4.16 |
| BEA      | 25 | 50.33 ± 4.04* | 71.67 ± 8.50* |
| 50       | 45.00 ± 2.00* | 55.33 ± 2.51* |
| 75       | 45.33 ± 4.72 | 49.67 ± 2.52 |
| LEA      | 25 | 60.67 ± 8.08* | 70.33 ± 8.38* |
| 50       | 49.00 ± 6.93* | 54.00 ± 5.57 |
| 75       | 48.33 ± 7.02 | 47.33 ± 3.78 |
| REA      | 25 | 51.67 ± 9.61 | 70.00 ± 7.81* |
| 50       | 45.00 ± 7.81 | 53.33 ± 5.13 |
| 75       | 50.00 ± 8.01 | 46.67 ± 4.16 |
| BC       | 25 | 51.00 ± 4.58* | 66.67 ± 7.50* |
| 50       | 47.00 ± 6.56 | 58.00 ± 8.18 |
| 75       | 45.67 ± 5.13 | 53.33 ± 4.93 |
| LC       | 25 | 61.33 ± 7.64 | 68.67 ± 7.64* |
| 50       | 51.67 ± 5.69* | 61.00 ± 4.58* |
| 75       | 48.33 ± 8.03 | 52.33 ± 4.04 |
| RC       | 25 | 51.67 ± 9.29 | 67.33 ± 6.81* |
| 50       | 46.00 ± 7.81 | 56.00 ± 7.81 |
| 75       | 41.33 ± 3.21 | 51.00 ± 7.55 |
| BPE      | 25 | 53.00 ± 6.24* | 70.33 ± 8.50* |
| 50       | 47.67 ± 3.51* | 64.00 ± 7.21* |
| 75       | 46.33 ± 5.51 | 51.00 ± 3.60 |
| LPE      | 25 | 61.00 ± 7.55* | 72.33 ± 6.51* |
| 50       | 53.67 ± 5.69* | 69.00 ± 7.55* |
| 75       | 49.33 ± 8.18 | 54.33 ± 2.08* |
| RPE      | 25 | 56.67 ± 5.69* | 67.00 ± 6.56* |
| 50       | 46.67 ± 7.23 | 62.00 ± 5.29* |
| 75       | 49.33 ± 7.37* | 56.67 ± 7.23 |

Values are expressed as mean ± S.E.M (n = 3). Data were analyzed by ANOVA followed by Dunnett’s *t*-test, *p < 0.01.
found that they would be a great source to exploit new pharmaceuticals.

The rates of morbidity and mortality are noteworthy in developed countries due to different thromboembolic disorders such as pulmonary emboli, deep vein thrombosis, strokes, and heart attacks. Different atherothrombotic ailments such as myocardial or cerebral infarction may develop due to the growth of a thrombus that interrupts the blood flow through vessels. Even, sometimes in critical conditions, patients die due to embolism [35, 36]. Currently, numerous thrombolytic agents are clinically employed to dissolve the clots that have already formed in the blood vessels. Although some are natural medications and others are modified recombinant technology products to make them more effective and site-specific, they all have several life-threatening complications [37, 38] which inspired the scientists to search for a newer, safer, and effective thrombolytic remedy such as plant-derived drugs. Furthermore, recent epidemiologic studies with plant and natural products experimentally proved that natural thrombolytic/fibrinolytic agents have the competence to lessen the risk of thrombosis more than the synthetic ones [38, 39]. During a comparative study between positive and negative controls, we noticed that clot lysis did not occur when water was added to the clot; whereas, the addition of different fractions of the extract revealed significant clot lysis. Among the different fractions, LE exhibited the highest clot lysis (30.77 ± 4.09%). This activity may be owing to the presence of different bioactive components like alkaloids, flavonoids, and tannins which were previously claimed to possess clot lysis property [40].

Tissue protein denaturation is one of the most common causes of arthritic symptoms, which can result in the formation of autoantigens in some situations. Chemical exposure and heat can cause stress, which causes protein to denature and autoantigens to form, causing harm to the joint synovial membrane and cartilage. Denaturation of proteins can also be caused by changes in hydrogen, hydrophobic, disulphide, and electrostatic linkages [41, 42]. As a result, chemicals that limit protein denaturation would be advantageous in the creation of antiarthritic medications. In the current study, all extracts hindered BSA denaturation in dose-dependent manner. The utmost activity was noticed for chloroform extract of root at 500 μg/ml (63.28 ± 5.96%). The inclusion of flavonoids and phenolic chemicals, which have previously been shown by several researchers, is thought to be responsible for the crude extracts’ antiarthritic effect [43, 44].

Helminth infection is a severe problem in both humans and animals since it causes a chronic and debilitating illness that can lead to death and promote medication resistance in other diseases. Natural products such as medicinal plants must be studied to inhibit helminth infection because they produce novel bioactive compounds with no or few side effects, are easily accessible to people in developing countries, and, more importantly, have the best compatibility with human physiology than conventional drugs. Resistance to anthelmintics, toxicity, and increased concerns about medication residues in animal products have reigned interest in plant-based remedies [45, 46]. The outcomes of the anthelmintic test revealed that all the extracts possess moderate activity in dose-dependent manner. In this study, when we compared the time of paralysis and time of death of earthworms between plant extracts and standard, we observed that the result was nearly close to the reference drug. Potency of these extracts was inversely proportional to the time taken for paralysis/death of the worms. The ethanolic extract of bark was found to be most potent (paralysis at 39.67 ± 2.52 min and death at 49.67 ± 3.51 min) at a concentration of 75 mg/ml. Previous literature showed that tannins, alkaloids, and saponins possess anthelmintic properties [46, 47]. Tannin can attach to free proteins in the...
host animal’s gastrointestinal tract or glycoprotein on the parasite’s cuticle (earthworms) and cause death [48]. Sap-
ponins primarily work by irritating mucus membranes in a way that causes parasite death [47]. Additionally, tannins
and alkaloids have a direct impact on the survivability of helminths’ parasitic stages and nervous system [46]. In
phytochemical screening tests of extracts, we confirmed the abovementioned bioactive substances. As a result, our
findings explain the plant’s anthelmintic properties, although more research is needed to extract and describe the
active ingredient.

Herbal preparations have recently received much atten-
tion as insecticidal substances due to their considerable
potential. The mortality rate generated by each crude extract
increased in a dose-dependent manner, according to our
insecticidal investigation. We found that exposure time and
dose were important factors in susceptibility. Plant extracts
have previously been reported to have insect repellent,
antifeedant, sterilizing, and toxic effects due to the presence
of bioactive plant components, such as carbohydrates, sa-
ponis, phytosterol, phenol, flavonoids, and tannins, which
have larvicidal action [49, 50]. As a result, polysaccharides,
saponins, flavonoids, glycosides, and other secondary me-
tabolites of the researched plant could help to explain the
poisonous effect in the insects.

5. Conclusion

The current study assessed in vitro thrombolytic, antiar-
thritic, anthelmintic, and insecticidal activities of petroleum ether, chloroform, and ethyl acetate soluble fractions of
ethanolic extract (leaf, bark, and root) of H. fomes Buch.
Ham. In conclusion, it can be said that H. fomes has potent
thrombolytic, antiarthritic, anthelmintic, and insecticidal
activities based on the research. All the solvent fractions
except the ethyl acetate fraction of the bark of H. fomes
possess significant thrombolytic activity ($p < 0.01$). Chloro-
form extract of root showed the highest antiarthritic potentiality (63.28 ± 5.96% at 500 μg/ml). The most insecticidal
property was seen in the case of ethanolic extract of
leaves (73%). However, further research is required for
extensive chemical profiling to identify the bioactive com-
ponents, which is mandatorily needed to delineate the exact
mechanism of action of these bioactive crude fractions.

Data Availability

The data used to support this study are included within the
article and in the supplementary file.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Supplementary Materials

The supplementary file containing four tables (Tables S1–S4)
summarized the raw data involved in the study. (Supple-
mentary Materials)

References

[1] S. Pattanayak, “Alternative to antibiotics from herbal ori-
gin—outline of a comprehensive research project,” Current
Pharmacogenomics and Personalized Medicine, vol. 16, no. 1,
pp. 9–62, 2018.

[2] S. Snehad, M. J. Hassain, N. M. Irfan et al., “Renoprotection
of selected antioxidant-rich foods (water spinach and red grape)
and probiotics in gentamicin-induced nephrotoxicity and
oxidative stress in rats,” Life, vol. 12, no. 1, p. 60, 2022.

[3] D. Y. Lu and T. R. Lu, “Herbal medicine in new era,” Hospice
and Palliative Medicine International Journal, vol. 3, no. 4,
pp. 125–130, 2019.

[4] T. Jannat, M. J. Hassain, A. M. El-Shehawi et al., “Chemical
and pharmacological profiling of Wrightia cucuccina (roxb.
Ex hormen.) sims focusing antioxidant, cytotoxic, antiar-thritic,
hypoglycemic, and analgesic properties,” Molecules, vol. 27,
no. 13, Article ID 4024, 2022.

[5] D. J. Newman and G. M. Cragg, “Natural products as sources
of new drugs over the 30 years from 1981 to 2010,” Journal
of Natural Products, vol. 75, no. 3, pp. 311–335, 2012.

[6] WHO, Traditional Medicines: Global Situation, Issues and
Challenges, World Health Organization, Geneva, Switzerland,
2011.

[7] S. A. Baba, M. Vahedi, I. Ahmad et al., “Crocus sativus L. Tepal
extract induces apoptosis in human U87 glioblastoma cells,”
BioMed Research International, vol. 2022, Article ID 4740246,
2022.

[8] S. Mitra, M. S. Lami, T. M. Uddin et al., “Prospective mul-
tifunctional roles and pharmacological potential of dietary
flavonoid narirutin,” Biomedicine and Pharmacotherapy,
vol. 150, Article ID 112932, 2022.

[9] N.-E. Tabassum, R. Das, M. S. Lami et al., “Ginkgo biloba: a
treasure of functional phytochemicals with multimedicinal
applications,” Evidence-based Complementary and Alternative
Medicine, vol. 2022, pp. 1–30, 2022.

[10] D. Arya and V. Patni, “Pharmacognostic profile and phyto-
chemical investigation of Pluchea lanceolata oliver and hiern.
in vivo and in vitro,” International Journal of Pharmaceutical
Sciences Review and Research, vol. 22, no. 2, pp. 157–161, 2013.

[11] M. C. S. Khutan, M. A. Muhit, M. J. Hassain, M. A. Al-
Mansur, and S. A. Rahman, “Isolation of phytochemical
constituents from Stevia rebaudiana (Bert.) and evaluation of
their antitumor, antimicrobial and antioxidant properties via
in vitro and in silico approaches,” Heliyon, vol. 7, no. 12,
Article ID e08475, 2021.

[12] N. Anjum, M. J. Hassain, M. R. Haque, A. Chowdhury,
M. A. Rashid, and M. R. Kuddus, “Phytochemical investiga-
tion of schleicheria oleosa (lour.) oken leaf,” Bangladesh
Pharmaceutical Journal, vol. 24, no. 1, pp. 33–36, 2021.

[13] R. Das, S. Mitra, A. M. Tareq et al., “Medicinal plants used
against hepatic disorders in Bangladesh: a comprehensive
review,” Journal of Ethnopharmacology, vol. 282, Article ID
114588, 2022.

[14] A. I. Sajib, S. M. R. Dewan, A. Das et al., “In vitro antimicro-
bial activity study and in vivo antiemic, antinociceptive
activity evaluation of leaves extract of Erioglossum rubig-
nosum using experimental animal model,” Oriental Pharmacy
Experimental Medicine, vol. 15, no. 2, pp. 135–140, 2015.

[15] A. J. Chakraborty, T. M. Uddin, B. M. R. Matin Zidan et al.,
“Allium cepa: a treasure of bioactive phytochemicals with
prospective health benefits,” Evidence based Complementary
and Alternative Medicine, vol. 2022, pp. 1–27, 2022.
[16] N. A. Masondo, G. Stafford, A. O. Aremu, and N. P. Makungu, “Acetylcholinesterase inhibitors from southern African plants: an overview of ethnobotanical, pharmacological potential and phytochemical research including and beyond Alzheimer’s disease treatment,” South African Journal of Botany, vol. 120, pp. 39–64, 2019.

[17] M. A. Hossain, S. Panthi, M. Asadujjaman, S. A. Khan, F. Ferdous, and S. K. Sadhu, "Phytochemical and pharmacological assessment of the ethanol leaves extract of Heritiera fomes Buch. Ham. Family Sterculiaceae," Journal of Pharmacognosy and Phytochemistry, vol. 2, no. 3, pp. 95–101, 2013.

[18] I. Mahmud, M. K. Islam, S. Saha et al., “Pharmacological and ethnomedical overview of Heritiera fomes: future prospects,” International Scholarly Research Notices, vol. 2014, Article ID 938543, 2014.

[19] C. Kalyani, C. S. L. Naga Tulasi, S. M. Sudarshan, A. Geetha, M. Lakshmi Narasu, and L. Saida, “Screening of antimicrobial and antioxidant activity of acetone extracts of Heritiera fomes whole plant against pathogens,” International Journal of Pharmaceutical Investigation, vol. 10, no. 4, pp. 564–568, 2020.

[20] J. K. Patra and H. Thatoi, “In-vitro bioactive potential of an ethnomedicinal mangrove plant (Heritiera fomes Buch. Ham.) from Odisha Coast, India,” Journal of Geo-Marine Sciences, vol. 44, no. 5, pp. 704–713, 2015.

[21] M. Rahmatullah, M. Ali, K. Nahar, M. Sinta, H. N. Khaleque, and F. J. Jahan, “An evaluation of anti-hyperglycemic and antiinflammatory effect of methanol extract of Heritiera fomes Buch-Ham. (Sterculiaceae).” bars in Swiss Albino mice,” Advances in Natural and Applied Sciences, vol. 5, no. 2, pp. 116–121, 2011.

[22] S. A. Rahman, M. M. Rahman, M. A. Hossain, and M. A. Rashid, “Chemical and biological investigations of leaves of abroma augusta linn,” Bangladesh Pharmaceutical Journal, vol. 19, no. 2, pp. 233–236, 2016.

[23] Y. Kc, R. Rai, N. Katuwal et al., “Phytochemicals, nutritional, antioxidant activity, and sensory analyses of Moringa oleifera Lam. collected from mid-hill region of Nepal,” Natural Product Research, vol. 36, pp. 470–473, 2020.

[24] F. Tabassum, S. H. Chadni, K. N. Mou, K. M. I. Hasif, T. Ahmed, and M. Akter, “In-vitro thrombolytic activity and phytochemical evaluation of leaf extracts of four medicinal plants of Astereaceae family,” Journal of Pharmacognosy and Phytochemistry, vol. 6, no. 4, pp. 1166–1169, 2017.

[25] R. Naz, H. Ayub, S. Nawaz et al., “Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan,” BMC Complementary and Alternative Medicine, vol. 17, no. 1, p. 302, 2017.

[26] S. B. Gopalakrishnan, K. Thangaraj, and E. Vadivel, "In vitro anthelmintic screening comparison of various crude extracts of the fruits of Cucumis trigonus roxb. and Cucumis sativus linn. Linn," World Journal of Pharmaceutical Research, vol. 3, no. 4, pp. 582–590, 2014.

[27] V. K. Lakshmi, K. B. Triveni, S. Anitha, and S. Shashidhara, “In vitro anthelmintic activity of Rotula aquatic Lour bark,” Pharma. Science.Monitor, vol. 3, pp. 2332–2339, 2012.

[28] N. Albariman, S. F. Sabran, N. Othman, N. Ishak, A. S. Dheyab, and N. Anjir, “Insecticidal and repellent activities of southeast asian plants towards insect pests: a review,” Asian Journal of Chemistry, vol. 32, no. 5, pp. 1026–1032, 2020.

[29] N. Jadid, E. Kurniawan, C. E. S. Himayani et al., “An ethnobotanical study of medicinal plants used by the Tengger tribe in Ngadisari village, Indonesia,” PLoS One, vol. 15, no. 7, Article ID e0235886, 2020.

[30] A. W. K. Yeung, M. Heinrich, A. Kijooa, N. T. Tzetkov, and A. G. Atanassov, “The ethnopharmacological literature: an analysis of the scientific landscape,” Journal of Ethnopharmacology, vol. 250, Article ID 112414, 2020.

[31] A. V. Barbosa, F. H. Silva, R. A. Q. C. Sá et al., “Use of medicinal plants in the diabetes treatment,” Journal of Herbal Medicine Research, vol. 5, p. 43, 2020.

[32] C. Rempel, M. J. Maciel, P. C. Bergmann, A. P. d. B. Morás, and C. Goettens, “Efeito antimicrobiano de plantas medicinais: uma revisão de estudos científicos,” Revista IberoAmericana de Ciências Ambientais, vol. 10, no. 4, pp. 57–82, 2019.

[33] M. Yu, I. Gouvinhos, J. Rocha, and A. I. R. N. A. Barros, “Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources,” Scientific Reports, vol. 11, no. 1, 2021.

[34] M. H. Sakib, A. A. Mahmood, L. K. Shill, and T. Barman, “Study on in-vitro thrombolytic activity of chloroform extract of leaves of Leucas zeylanica,” Journal of Scientific Research and Advances, vol. 2, no. 4, pp. 141–143, 2015.

[35] A. S. Apu, F. A. Chowdhury, F. Khatun, A. T. Jamaluddin, A. Pathan, and A. Pal, “Phytochemical screening and in vitro evaluation of pharmacological activities of Aphanamixis polystachya wall parish paper fruit,” Tropical Journal of Pharmaceutical Research, vol. 12, no. 1, pp. 111–116, 2013.

[36] S. Rajeswari and R. Vidhya, “Evaluation of in vitro thrombolytic and antiplatelet activities of Wedelia trilobata (linn.),” Innovare Journal Of Life Sciences, vol. 5, no. 3, pp. 6–10, 2017.

[37] M. R. Chowdhury, A. R. Islam, and M. A. G. Mukstadir, “Thrombolytic activity of lagerstroemia speciosa leaves,” Discovery Phytomedicine, vol. 4, no. 4, p. 41, 2017.

[38] M. Rahman, M. Rahman, M. Chowdhury, M. F. Islam, and S. Barua, “Antidiarrheal and thrombolytic effects of methanol extract of Wilstroemia indica (L.) C. A. Mey leaves,” International Journal of Green Pharmacy, vol. 9, no. 1, p. 8, 2015.

[39] M. S. Uddin, M. S. Millat, M. S. Islam et al., “Exploration of in vivo thrombolytic, antiplatelet, cytoxic and in vivo antithrombotic potentials with phytochemical screening of flowers of Brassica nigra,” Future Journal of Pharmaceutical Sciences, vol. 6, no. 1, p. 73, 2020.

[40] N. Hoque, M. Z. Imam, S. Akter et al., “Antioxidant and antihyperglycemic activities of methanolic extract of Glinus oppositifolius leaves,” Journal of Applied Pharmaceutical Science, vol. 1, no. 7, pp. 50–53, 2011.

[41] E. Umaphaty, E. J. Ndebia, A. Meeme et al., “An experimental evaluation of Albua setosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation,” Journal of Medicinal Plants Research, vol. 4, no. 5, pp. 789–795, 2010.

[42] S. S. Vulluri, S. R. Bammidi, S. C. Chippada, and V. Meena, “In-vitro anti-arthritis activity of methanolic extract of Bacopa monniera,” IJCEPR, vol. 2, pp. 156–159, 2011.

[43] J. S. Deng, C. S. Chi, S. S. Huang, P. H. Shie, T. H. Lin, and G. J. Huang, “Antioxidant, analgesic, and anti-inflammatory activities of the ethanolic extracts of Taxillus liquidambaricola,” Journal of Ethnopharmacology, vol. 137, no. 3, pp. 1161–1171, 2011.

[44] U. H. Hasan, “Antiarthritic efficacy of clematis orientalis,” Bangladesh Journal of Pharmacology, vol. 13, no. 2, pp. 142–148, 2018.
[45] A. Paul, M. Adnan, M. Majumder et al., "Anthelmintic activity of *Piper sylvaticum* Roxb. (family: piperaceae): in vitro and in silico studies," *Clinical Phytoscience*, vol. 4, no. 1, p. 17, 2018.

[46] S. Zenebe, T. Feyera, and S. Assefa, "In vitro anthelmintic activity of crude extracts of aerial parts of *Cissus quadrangularis* L. and leaves of *Schinus Molle* L. against *Hae- monchus contortus*," *BioMed Research International*, vol. 2017, Article ID 1905987, 2017.

[47] A. B. Maicale, S. L. Attimarad, D. S. Haradagatti, and A. Karigar, "Anthelmintic activity of fruit pulp of cordial dichotoma," *International Journal of Research in Ayurveda and Pharmacy*, vol. 1, no. 2, pp. 597–600, 2010.

[48] K. Gnaneswari, Y. Padma, R. R. Venkata, and K. N. Jayaveera, "In vitro anthelmintic activity of *Leonotis nepetifolia* (L.) R. Br., a potential medicinal plant," *Journal of Chemical and Pharmaceutical Research*, vol. 5, no. 2, pp. 345–348, 2013.

[49] C. H. Liu, A. K. Mishra, R. X. Tan, C. Tang, H. Yang, and Y. F. Shen, "Repellent and insecticidal activities of essential oils from *artemisia princeps* and *cinnamomum camphora* and their effect on seed germination of wheat and broad bean," *Bioresource Technology*, vol. 97, no. 15, pp. 1969–1973, 2006.

[50] R. Uncini Manganelli, L. Zaccaro, and P. E. Tomei, "Antiviral activity in vitro of *Urtica dioica* L., *Parietaria diffusa* M. et K. and *Sambucus nigra* L," *Journal of Ethnopharmacology*, vol. 98, no. 3, pp. 323–327, 2005.