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Exploring therapeutic potential of *Woodfordia fruticosa* (L.) Kurz leaf and bark focusing on antioxidant, antithrombotic, antimicrobial, anti-inflammatory, analgesic, and antidiarrheal properties

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Abstract

Background and Aims: The study aimed to evaluate the pharmacological properties of methanolic extracts of leaves and barks of *Woodfordia fruticosa* (L.) Kurz (family: Lythraceae) focusing on antioxidant, thrombolytic, anti-inflammatory, antibacterial, analgesic, and antidiarrheal effects.

Methods: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay, clot lysis, disc diffusion, and membrane stabilizing methods were employed to assess in vitro antioxidant, thrombolytic, antibacterial, and anti-inflammatory properties of the leaf and bark methanolic extracts (ME) of *W. fruticosa* and different organic solvents, that is, petroleum ether (PE), dichloromethane (DCM), chloroform (CL), and aqueous (AQ) fractions. In addition, *in vivo* central and peripheral analgesic and antidiarrheal activities of both crude extracts were evaluated at two doses (200 and 400 mg/kg of body weight [bw]).

Results: All the extracts and fractions showed promising antioxidant properties by scavenging DDPH free radicals with IC_{50} of 6.11–20.79 µg/mL. AQ fraction (41.24%) of leaves and ME (44.90%) of bark exerted notable in vitro thrombolytic activity. The CL fraction of leaves and AQ fraction of the bark showed 43.16% and 45.37% inhibition of RBC hemolysis, respectively, compared to the inhibition of RBC hemolysis by aspirin in a hypotonic-induced membrane stabilizing assay. Besides, both extracts were observed to provide significant ($p < 0.001$) central and peripheral analgesic responses at both doses of 200 and 400 mg/kg bw. Furthermore, both doses of bark extract ($p < 0.001$) and the 400 mg/kg bw of leaf extract ($p < 0.05$) were observed to possess statistically significant antidiarrheal activity. Additionally, in an *in vivo* acute toxicity investigation, both extracts had a median lethal dose (LD_{50}) greater than 5000 mg/kg bw, indicating their safety level.

Conclusion: The current study proves the ethnomedicinal uses of *W. fruticosa*; however, further studies are required for phytochemical screening to isolate the responsible bioactive compounds and discover the lead molecules from the plant species.

KEYWORDS

analgesics, antidiarrheal, antimicrobial, antioxidant, membrane stabilization, thrombolytic, *Woodfordia fruticosa*

1 | INTRODUCTION

Since ancient times, medicinal plants have been used for treating human diseases, and their use continues to be prevalent in modern times. Plant-based remedies are extensively engaged in managing conditions such as inflammatory disorders, central nervous system diseases, cancer, viral infections, fungal ailments, and various other health issues.[1] Through experimental and clinical investigations, the use of natural products and herbal extracts for treating human diseases and disorders has finally begun to get scientific acceptance.[2] Most developing countries, particularly those in Asia, Africa, Latin America, and the Middle East, rely on traditional medicine, including herbal medicines, to fulfill the primary healthcare requirements and concerns of the rural people.[3] According to the World Health Organization (WHO), nowadays, over 80% of people living in rural areas turn to medicinal plants as an alternative approach to their basic healthcare needs.[4] This preference likely stems from the fact that these plants offer natural antioxidants, have minimal or no harmful side effects, are cost-effective, and can contribute to longer life spans while effectively treating a range of illnesses.[4] Significant biological complications may occur due to drug-induced toxic effects, resulting in fatality. As a result, most medications with toxicological attributes have been dragged from the market. Plant-based therapies or herbal treatments have recently gained popularity and significance due to their medicinal properties, purity, and cost-effectiveness.[5,6]

Woodfordia fruticosa (L.) Kurz (Syn. *Lythrum fruticosum* L.) has been extensively utilized as a traditional medicine in many Southeast Asian nations. *W. fruticosa*, commonly recognized as a fire-flame bush in English and Dhaiphul in Bengali, is a species of the Lythraceae family.[7] This family consists of approximately 27 genera and nearly 575 species, with the majority found in tropical countries and only a few in temperate regions.[8] The plant is extensively found in India, Pakistan, Malaysia, Indonesia, Sri Lanka, China, Japan, and in tropical regions of Africa.[9] In Bangladesh, the family Lythraceae is represented by six genera and 24 species, and only *W. fruticosa* occurs in hill tracts of Chittagong, Cox's Bazar, and Dhaka districts.[8] The traits of this plant species include large shrub, pendulous branches, and pubescent when it is young.

Numerous investigations comprising the structural elucidation of the phytochemicals of *W. fruticosa* utilized the flowers, with few studies employing the stems and leaves. In 2022, Mishra et al. confirmed the occurrence of alkaloids, steroids, tannin, and saponins in leaf extract.[10] *W. fruticosa* highly comprises phenolics, chiefly hydrolyzable tannins, and flavonoids. In addition, gallic acid, ellagic acid, and C-glycoside of gallic acid were found in leaves, stems, and flowers. Triterpenoids, that is, lupeol, betulin, betulinic acid, oleanolic acid, and β-sitosterol, are present in the stems, flowers, and leaves.[10–13]

W. fruticosa has long been employed as traditional medicine by medical practitioners in the Southeast Asian region, especially in India. Ayurvedic and Unani medicinal systems substantially use the flower part of the plant as an herbal remedy.[14,15] The flower has been used to cure fever, dysentery, open wounds, leprosy, rheumatism, blood diseases, menorrhagia, leucorrhea, and toothache.[15] A series of pharmacological studies were performed to justify and scientifically prove the traditional uses of this plant species. Evidence revealed the significant host-mediated antitumor activities, antioxidant, antibacterial, and analgesic activities of the flower, essential oil, and leaf extracts of *W. fruticosa*.[15–23] Despite having scientific verification of the traditional uses, mainly of this plant's flower and leaf parts, more investigation into the pharmacological activities of the bark of *W. fruticosa* is needed. Thus, the present study emphasized the evaluation of *in vitro* and *in vivo* bioactivities of the leaf and bark extracts of *W. fruticosa*, focusing on antioxidant, thrombolytic, antimicrobial, antibacterial, analgesic, and antidiarrheal properties. In addition, the study measured the median lethal dose (LD_{50}) of the leaf and bark extracts by utilizing an *in vivo* acute oral toxicity study to ensure the safety level of the plant species.

2 | MATERIALS AND METHODS

2.1 | Plant materials

Both the leaves and barks of *W. fruticosa* (L.) Kurz were collected from the Chapainawabganj district of Bangladesh in November 2019. Based on its morphological characteristics, Dr. Nahid Sultana, Department of Botany, Jagannath University, and Dr. M. Oliur Rahman, Department of Botany, University of Dhaka, Dhaka, Bangladesh, identified the species and validated both the leaves and barks of the collected plant sample. The Bangladesh National Herbarium in Dhaka, Bangladesh, has formally recorded and archived a voucher specimen with the accession number DACB 88284.

2.2 | Extraction and fractionation

After the collection, clean water was used to instantly wash away dirt and undesirable elements from the collected leaves and barks. After being thoroughly cleaned, the samples were left out in the open sunlight for several days to allow the leaves and barks to dry. After that, the dried leaves and bark were ground into a coarse powder using a high-capacity mill. It is noted that 500 g of each sample was used for the "solid-liquid" extraction technique, which involved soaking them in 2.5 L of methanol. The extraction process was allowed for 2 weeks at room temperature, with periodic shaking and stirring. The extracts underwent filtration using a clean cotton mass and Whatman filter paper number 1. A rotary evaporator was employed to condense the filtrates at a temperature lower than 40°C and a pressure of around 337 mbar. Then, 5 g of each of the crude extract in methanol (ME) was taken to fractionate using different solvents, such as petroleum ether (PE), dichloromethane (DCM), chloroform (CL), and aqueous (AQ) utilizing the protocol known as modified Kupchan partitioning process.[24] The obtained fractions from 5 g of crude extracts are stated in Table 1. Finally, the subsequent partitions were condensed to yield dry soluble materials.

TABLE 1 Kupchan partitions of leaves and bark of *Woodfordia fruticosa*.

Fractions	Code of extract	Leaves (g)	Barks (g)
Methanolic crude extract	ME	5.00	5.00
Pet-ether soluble fraction	PE	0.78	0.82
Dichloromethane soluble fraction	DCM	0.48	0.53
Chloroform soluble fraction	CL	0.40	0.35
Aqueous soluble fraction	AQ	2.95	2.98

Abbreviations: AQ, aqueous soluble fraction; CL, chloroform soluble fraction; DCM, dichloromethane soluble fraction; ME, methanolic extract; PE, pet-ether soluble fraction.

2.3 | Drugs and chemicals

In this study, analytical graded reagents were used. Methanol, dichloromethane, chloroform, petroleum ether, Folin–Ciocalteu reagent, sodium carbonate, 1,1-dipheny-l-2-picrylhydrazyl (DPPH), potassium dichromate, ascorbic acid (AA), gallic acid (GA), dimethyl sulfoxide (DMSO), Tween-80, glucose, and castor oil were purchased from BDH Chemicals. The saline water was obtained from Beximco Infusion Ltd. Furthermore, morphine (manufactured by Gonoshastho Pharmaceuticals Ltd.), diclofenac sodium, glibenclamide, and loperamide (supplied by Square Pharmaceuticals Ltd.) were also used.

2.4 | *In vivo* study design

A total of 48 Swiss Albino mice were divided randomly into eight groups, with six mice randomly selected in each group ($n = 6$, three male and three female mice), for the in vivo experiments. The following section provided an explanation for this sample size and how it was determined. The groups were categorized as negative control, positive control, and four test groups. The test groups received methanolic extracts of leaves and bark at doses of 200 (MEL 200 and MEB 200) mg/kg bw and 400 (MEL 400 and MEB 400) mg/kg of bw. Male and female Swiss Albino mice, aged 4–5 weeks and weighing between 25 and 35 g, were used for the tests. The investigation utilized only healthy and fresh mice; none of them were sick. The mice were housed in accordance with the guidelines set by the Federation of European Laboratory Animal Science Associations (FELASA) at the State University of Bangladesh animal housing facility. The housing conditions included a temperature of 24.0 ± 1°C, relative humidity of 55%–65%, and a 12-h light/12-h dark cycle. The mice were acclimatized to the environment for a week before the experiments. They were fed rodent feed purchased from the ICDDR,B (International Centre for Diarrhoeal Disease Research, Bangladesh. The ethical guidelines and protocols of the study underwent a comprehensive evaluation by the Animal Ethics Committee at the State University of Bangladesh, resulting in their thorough review and approval. In addition, the *in vivo* research was conducted according to the updated animal research: reporting *in vivo* experiments (ARRIVE) guidelines,[25] and the manuscript was prepared by following the author checklist of the ARRIVE guidelines 2.0 (Supporting Information).

2.5 | Sample size determination for *in vivo* study

Estimating the optimum sample size is critical when designing research in animal model. Selecting too few animals may result in the missing of major population variations, while choosing too many may result in resource waste and ethical problems. We used the "resource equation" method[26] for estimating the sample size because we were unable to determine the standard deviation and effect size. As part of the sample size determination procedure, this method determines an acceptable range for error degrees of freedom (DF) within an analysis of variance (ANOVA). When dealing with one-way ANOVA in this case, we can estimate the sample size for the error degrees of freedom related to between-subjects variability (also known as within-subject degrees of freedom) as follows:

$$n = \mathrm{DF}/k + 1.$$

Here, n denotes the number of animals in each group, DF for degrees of freedom, and k for the overall number of groups. The allowable range of the DF to establish the minimum and maximum number of animals per group was considered. Using the DF value's minimum (10) and maximum (20) values, we substituted them into our equations.[26] In this instance, we removed the normal control and positive control groups by setting the value of k is 2, which

represents two test groups for each extract (200 and 400 mg/kg bw). Therefore, we determined that the minimum and maximum numbers of animals per group should be roughly 6 ($10/k + 1 = 10/2 + 1$) and 11 ($20/k + 1 = 20/2 + 1$), respectively. We adjusted the number of animals per group as necessary to maintain the DF within predetermined limits. The use of animals in this study was rigorously governed by the "3R" (replacement, reduction, and refinement) principles, which are set down in international and Swiss regulations.[27] In accordance with these ethical guidelines, we conducted this exploratory study on the fewest possible animals ($n = 6$). The execution of the entire investigation was done with the utmost adherence to these ethical principles.

2.6 | Antioxidant activity

2.6.1 | Total phenolic content

Total phenolic content (TPC) of the extractives was calculated using the method developed by Harbertson and Spayd.[28] To create the mixtures, 0.5 mL of sample solutions with a concentration of 2 mg/mL were combined with 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times in water) and 2.0 mL of 7.5% (w/v) Na_2CO_3 solution in each test tube. The solutions were then incubated for 20 min at room temperature. After the incubation period, the absorbance at 760 nm was measured using a UV spectrophotometer, and the TPC of the samples was measured by preparing a standard curve using gallic acid solutions with varying concentrations. The phenolic contents of the samples were expressed in milligrams of gallic acid equivalent (GAE) per gram of the extract.

2.6.2 | DPPH free radical scavenging assay

The antioxidant activity of the test samples was evaluated using a stable free radical DPPH. This assessment measures the samples' capacity to scavenge DPPH and compares it to a traditional antioxidant known as butylated hydroxytoluene (BHT).[29] To perform this assay, solutions of the samples, either extractives or control, were prepared at various concentrations ranging from 500 to 0.977 µg/mL. A total of 2 mL of these solutions were mixed with 3.0 mL of a methanol solution containing DPPH (20 µg/mL) and left in a dark room at room temperature for 30 min. Subsequently, the mixture obtained was subjected to measurement of absorbance at a wavelength of 517 nm utilizing a UV spectrophotometer. The percentage inhibition (I%) of the DPPH free radical was determined by employing the formula stated below:

$$I\% = (1 - A_{sample}/A_{blank}) \times 100,$$

where A_{blank} = absorbance of the control solution, A_{sample} = absorbance of the samples.

The IC_{50} value, which represents the concentration at which 50% inhibition occurs, was determined by plotting the percentage of inhibition against the different concentrations of the extracts and calculating it.

2.7 | Thrombolytic activity

Blood clots or thrombus occludes the normal blood flow, resulting in an inadequate supply of blood and oxygen to cells and tissues.[30] Hence, the clot lysis activity of the plant has been evaluated in the current investigation. Fresh blood was drawn from a healthy human and placed in three sterile preweighed microtubes. Then, the blood samples were subjected to incubation for 45 min at 37°C. The upper fluid was completely exuded from all the tubes after thrombus formation. Afterwords, 100 µL of crude extracts and soluble fractions were added to each tube and again incubated for 90 min. Subsequently, the fluid released by the clot was removed, and the weights of the tubes were taken again to determine the differences in weights when the lump ruptured. In this study, the positive control was streptokinase, and the negative control was water (distilled). Clot lysis was reckoned in percentage as follows:

$$\%\ \text{of clot lysis} = [(\text{mass of clot after lysis}) / (\text{mass of clot before lysis})] \times 100\%.$$

2.8 | Antimicrobial screening

The effectiveness of the plant materials in fighting against microbes was evaluated using the disc diffusion method developed by Bauer et al.[31] In this method, small filter paper disks with a diameter of 6 mm were soaked in specific quantities of the test samples mixed with a nutrient agar medium. These disks were then evenly distributed on the agar surface along with the microorganisms being tested. A disc containing the antibiotic ciprofloxacin was included as a reference, and a blank disc soaked in solvent was used as a control. The plates were incubated at 37°C for a day (24 h) to ensure optimal diffusion of the test materials throughout the agar. The growth of microbes in the vicinity of the disks was inhibited by the antimicrobial properties of the test materials, resulting in clearly visible zones without microbial growth. Finally, the ability to kill microorganisms of the test agents was assessed by calculating the size of the zones where growth was inhibited, expressed in millimeters.[31,32]

2.9 | Anti-inflammatory activity

The crude methanolic extracts and the fractions of the leaves and bark of *W. fruticosa* were subjected to *in vitro* anti-inflammatory evaluation using the following established techniques described by Omale.[33]

2.9.1 | Hypotonic solution-induced hemolysis

A volume of 4.5-mL solution containing 50 mM hypotonic NaCl was combined with 10 mM phosphate-buffered saline (pH 7.4). To create a mixture, 0.50 mL of human blood suspension was added along with each plant extract at a concentration of 2.0 mg/mL, as well as the hypotonic solution. These mixtures were allowed to react for 10 min and then subjected to centrifugation at 3000 rpm for 10 min. The absorbance of the liquid at the top was measured at 540 nm. Acetyl salicylic acid (ASA) or aspirin at a concentration of 0.10 mg/mL was used as a standard. The percentage inhibition of hemolysis was determined as follows:

$$\% \text{ inhibition of hemolysis} = (A_{control} - A_{sample/standard}/A_{control}) \times 100\%,$$

where $A_{control}$ = absorbance of control (hypotonic and buffered saline solution), $A_{sample/standard}$ = absorbance of test sample and standard.

2.10 | Heat-induced hemolysis

Leaf and bark extracts and the fractions of *W. fruticosa* were subjected to heat-induced hemolysis by taking 5-mL isotonic buffer, 1.0 mg/mL of extractives, and 30-µL human blood in each tube, which were kept in two identical sets of tubes for centrifugation.[34] One set was placed in a water bath at 54°C, while the other set was placed in an ice bath at 0–5°C for 20 min. Afterward, the mixtures were centrifuged at 1300 rpm for 3 min, and the absorbance of the upper fluid was measured at 540 nm. A standard solution of acetylsalicylic acid (0.10 mg/mL) was used for comparison. The inhibition of hemolysis was determined as a percentage using the following formula:

$$\% \text{ inhibition of hemolysis} = 1 - (AH_{sample} - AU_{sample}/AH_{control} - AU_{sample}) \times 100\%,$$

where, AU_{sample} = absorbance of sample unheated, AH_{sample} = absorbance of sample heated, and $AH_{control}$ = absorbance of control solution heated.

2.11 | Central analgesic activity

The tail immersion scheme was applied to investigate the central analgesic activity.[35] The mice were given two doses of crude extracts of the leaves and bark (200 and 400 mg/kg of bw), and the tail end (around 5 cm) was submersed in hot water to induce pain. Tail immersion time was the time mice took to avert their tails after the extract or drug administration, and the response was determined at 30, 60, and 90 min. The positive control in the experiment involved using subcutaneous morphine at a dosage of 2 mg/kg bw. As for the negative control, a saline mixture containing oral 1% Tween 80 was administered at a rate of 0.1 mL per 10 mg.

2.12 | Peripheral analgesic activity

We used the acetic acid-induced writhing technique to evaluate the *W. fruticosa* leaf and bark extracts' effectiveness in pain relief at the peripheral level.[36] Intraperitoneal acetic acid (0.1 mL) was administered to all the mice for pain sensation. The positive control mice group received diclofenac sodium orally at a dose of 5 mg/kg bw. The experimental groups were given the crude extracts at two different doses. After administering the test materials, the number of writhing movements was recorded for 5 min. The percentage of writhing inhibition was then calculated by utilizing the following formula:

$$\text{Percentage of inhibition} = \frac{\text{Control writhing response} - \text{Test writhing response}}{\text{Control writhing response}} \times 100\%.$$

2.13 | Antidiarrheal activities

The antidiarrheal effect of *W. fruticosa* leaves and bark extracts was investigated in mice using the castor oil-induced diarrhea technique.[37] The study included a negative control group that received a 10 mL/kg bw solution of Tween 80, a positive control group that received a 50 mg/kg bw dose of loperamide, and test groups that were administered two different doses of crude extracts (200 and 400 mg/kg of bw). After a 30-min waiting period, all animals were given 1 mL of castor oil orally to induce diarrhea, following the established method. The number of fecal stools produced by each mouse was recorded hourly for a duration of 4 h. The antidiarrheal activity of the extracts was assessed by comparing the observations from the experimental groups to those of the negative control mice. The percentage of inhibition of diarrhea was calculated using the formula below as:

$$\% \text{ inhibition} = \frac{\text{Mean defecation of control} - \text{Mean defecation of test sample or standard}}{\text{Mean defecation of control}} \times 100\%.$$

2.14 | *In vivo* acute oral toxicity test

The study used 33 male and 33 female Swiss Albino mice (28–34 g) between the ages of 8 and 10 weeks for an acute oral toxicity test. The test was conducted in accordance with OECD (Organization for Economic Co-operation and Development) 423 guidelines.[38] The mice were separated into two groups based on their random selection: one group received merely vehicle (1% tween 80) as therapy, while the other received either leaf or bark extracts. The mice were given single doses of the leaf and bark extracts orally at doses ranging from 1000 to 5000 mg/kg, along with a control group that received a vehicle solution. This was done after the mice had fasted for the previous night. The mice were subsequently observed for 14 days. A total of 11 distinct groups, each with three male and three female mice, were as follows:

Group 1: A control group receiving 1% Tween 80 only (untreated),

Group 2: 1000 mg/kg bw of leaf extract,

Group 3: 2000 mg/kg bw of leaf extract,

Group 4: 3000 mg/kg bw of leaf extract,

Group 5: 4000 mg/kg bw of leaf extract,

Group 6: 5000 mg/kg bw of leaf extract,

Group 7: 1000 mg/kg bw of bark extract,

Group 8: 2000 mg/kg bw of bark extract,

Group 9: 3000 mg/kg bw of bark extract,

Group 10: 4000 mg/kg bw of bark extract,

Group 11: 5000 mg/kg bw of bark extract.

All the mice received a regular diet and water after 60 min of dosing. After administering the medication, the treated mice were observed for 30 min, then hourly for 8 hours, and afterwords daily for the following 14 days. Visual observations of mortality, behavioral patterns (for example, moving about, being sleepy, having convulsions), physical appearance changes (for example, being unable to move, body trembling), and injury were tracked throughout the testing period.

2.15 | Statistical analysis

The established procedures offered by Assel et al.[39] were followed for the data processing and interpretation of the findings. Graphs and data processing regarding the in vitro investigation were done by utilizing MS Excel (version 10.0). However, as the data from the in vitro tests were acquired from a single experiment, statistical analysis was not performed for the in vitro study. Furthermore, we conducted the statistical analysis for the data obtained from the in vivo tests by comparing the treated groups to the control (vehicle) group. The average values with their associated standard errors of mean were expressed as mean ± SEM to represent the results obtained from the in vivo evaluations. The data obtained from *in vivo* tests were analyzed using GraphPad Software. A one-way ANOVA was performed on all variables, followed by student's t-test. Any *p*-values below 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Antioxidant activity

The antioxidant activity of *W. fruticosa* was investigated by determining the total phenolic content and free radical scavenging activity using DPPH, as illustrated in Figures 1 and 2. The results revealed that the DCM soluble fraction of the leaf extract exhibited the highest phenolic substance content (76.20 mg of GAE/g of extractives), followed by the ME soluble fraction (46.33 mg of GAE/g of extractives) and the CL soluble fraction (40.83 mg of GAE/g of extractives). Among the bark extractives, the ME soluble fraction demonstrated the highest amount of phenolic derivatives (77.99 mg of GAE/g of extractives), followed by the DCM soluble fraction (49.26 mg of GAE/g of extractives) and the PE soluble fraction (36.45 mg of GAE/g of extractives). Furthermore, the DCM soluble fraction of the leaves (6.11 µg/mL) and the ME soluble fraction of the bark (6.07 µg/mL) exhibited the highest IC_{50} values in the free radical scavenging activity assay, surpassing the effectiveness of BHT (2.87 µg/mL), as depicted in Figure 2.

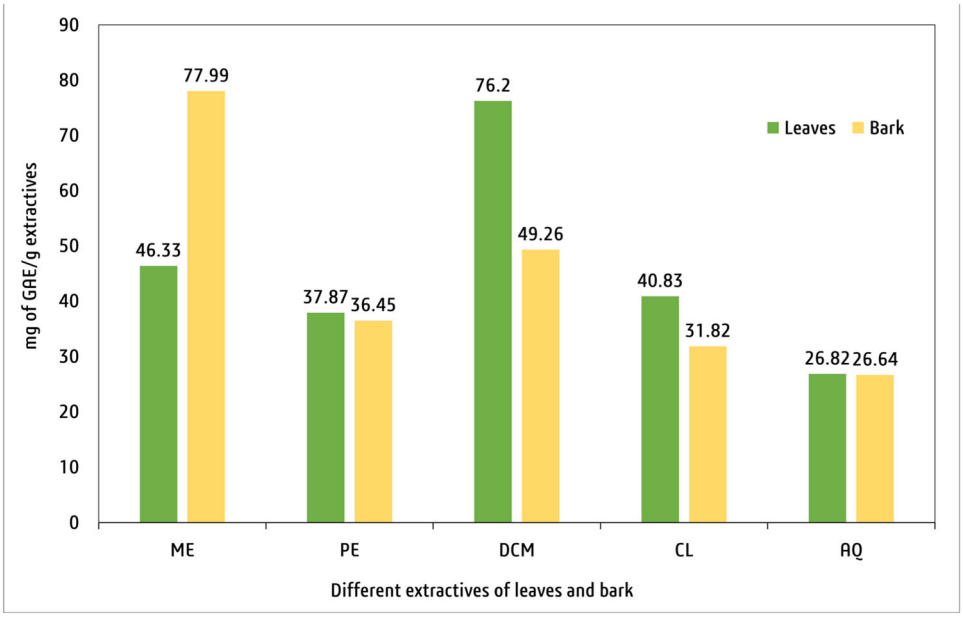

FIGURE 1 Total phenolic content (mg of GAE/g of extractives) of different extractives of leaves and bark of *Woodfordia fruticosa*. AQ, aqueous soluble fraction; CL, chloroform soluble fraction; DCM, dichloromethane soluble fraction; ME, methanolic extract; PE, pet-ether soluble fraction.

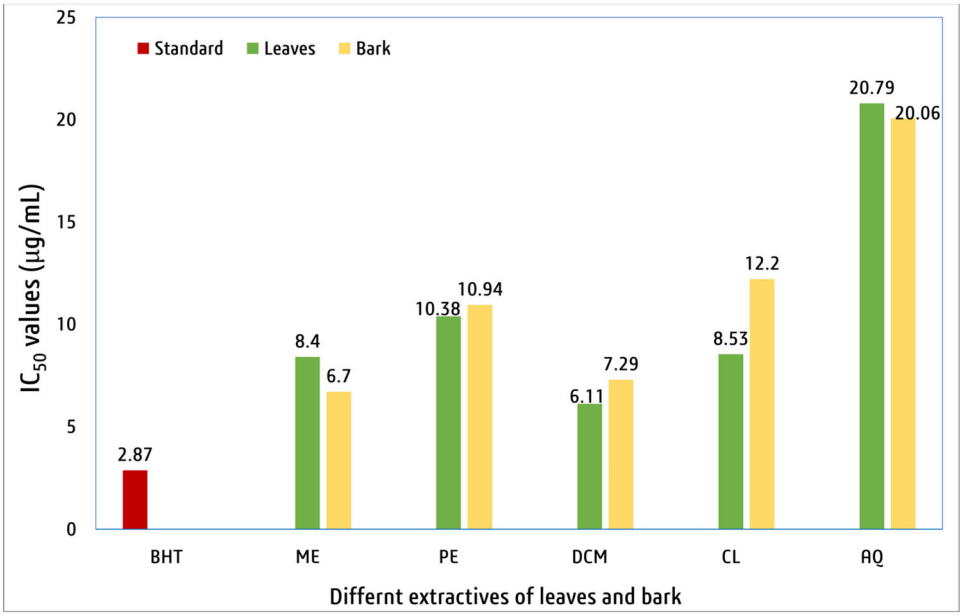

FIGURE 2 IC_{50} values of the standard and partitionates of leaves and bark of *Woodfordia fruticosa*. AQ, aqueous soluble fraction; BHT, butylated hydroxy toluene; CL, chloroform soluble fraction; DCM, dichloromethane soluble fraction; ME, methanolic extract; PE, pet-ether soluble fraction.

3.2 | Thrombolytic activity

Thrombolytic activities were assessed among methanol extract and its different partitionates as shown in Figure 3. All the fractions exhibited mild to moderate clot lysis properties. In case of leaves, the maximum activity was found in AQ fraction (41.24%), followed by CL (38.80%) and ME (38.22%). On the other hand, ME of bark possesses the highest percent clot lysis activity (44.90%), and the percentages of other fractions ranged from 23.41% (PE) to 39.12% (DCM).

3.3 | Antimicrobial properties

The antimicrobial effect of the leaves and bark of *W. fruticosa* was evaluated against different strains of bacteria and is presented in Table 2. The findings displayed that only PE of leaves showed activity against the gram-negative bacteria with inhibitory zone of 7 mm against *Escherichia coli* and *Shigella boydii*, and 10 mm against *Sh. dysenteriae*. On the contrary, all the fractions of bark except the aqueous soluble fraction exhibited notable activities against both gram-positive and gram-negative bacteria. DCM of bark displayed highest inhibitory zone against the growth of *S. boydii* (15 mm) followed by *Sarcina lutea* (14 mm), *Salmonella paratyphi* (12 mm). PE soluble fraction showed highest inhibition against *S. lutea* (12 mm) followed by *S. boydii* (10 mm) and *Salmonella paratyphi* (9 nm). In addition, CL soluble fraction inhibited the growth of *S. dysenteriae* (11 mm), *S. boydii*, and *S. paratyphi* (10 mm), and so on. Crude methanol extract possesses the potential to prevent the growth of *S. paratyphi* (11 mm), *S. dysenteriae* (8 mm), and so on.

3.4 | Anti-inflammatory activity

CL of leaves and AQ of the bark of *W. fruticosa* revealed maximum inhibition of RBC hemolysis by 43.16% and 45.37%, respectively, and the values were compared to the inhibition by acetylsalicylic acid (ASA, 0.10 mg/mL) (78.30%) in hypotonic solution-induced hemolysis as shown in Figure 4. On the other hand, the ME of leaves and DCM of bark demonstrated maximum inhibition of 31.73% and 26.53%, respectively, in comparison with 42.12% inhibition by ASA in heat-induced hemolysis of RBC, as shown in Figure 5.

3.5 | Central analgesic activity

The crude extracts of leaves and bark (200 and 400 mg/kg bw) of *W. fruticosa* were investigated for central analgesic activities (Table 3) employing the tail immersion method in mice. The extracts revealed notable central analgesia by elongating the time to deflect the tails of mice from heat, which was statistically significant ($p < 0.001$). Two doses of leaf extract increased the time of tail immersion up to 9.00 ± 0.13 and 9.23 ± 0.13 min, respectively, whereas 7.99 ± 0.34 and 9.45 ± 0.07 min time of tail immersion were observed in bark extract, respectively, after 90 min of drug loading. Morphine increases the deflection time up to 13.32 ± 0.39 min.

3.6 | Peripheral analgesic activity

W. fruticosa leaves and bark extract were investigated for peripheral analgesic activity by acetic acid-induced writhing technique, and the

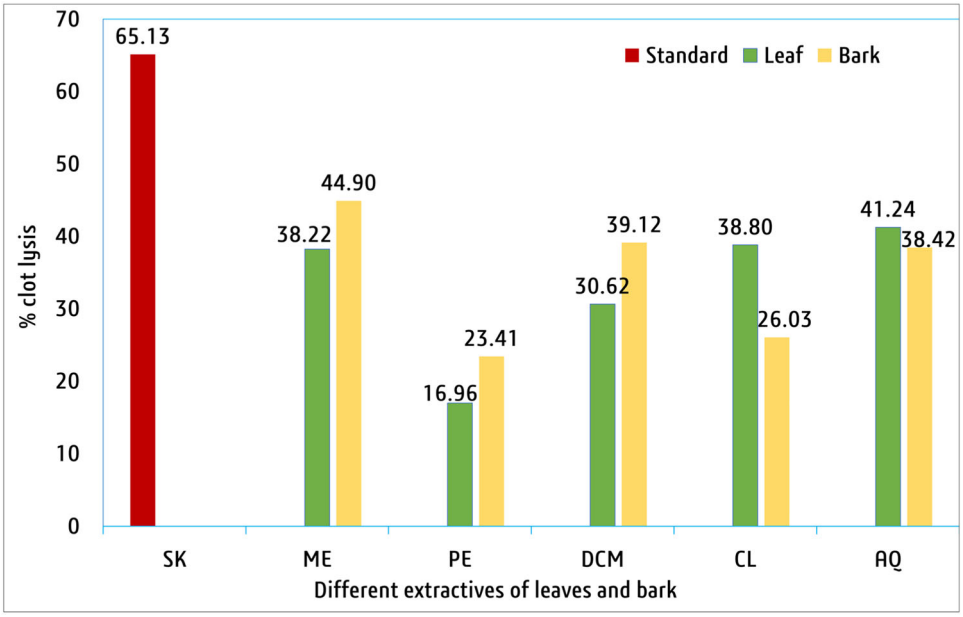

FIGURE 3 Thrombolytic activity of the leaves and bark of *Woodfordia fruticosa*. AQ, aqueous soluble fraction; CL, chloroform soluble fraction; DCM, dichloromethane soluble fraction; ME, methanolic extract; PE, pet-ether soluble fraction; SK, streptokinase.

TABLE 2 Antimicrobial screening with zone of inhibition (mm) of test materials of leaves and bark of *Woodfordia fruticosa*.

Test organisms	STD	Leaf extracts					Bark extracts				
		ME	PE	DCM	CL	AQ	ME	PE	DCM	CL	AQ
Gram-positive bacteria											
Bacillus cereus	45	0	0	0	0	0	0	0	0	0	0
Bacillus megaterium	38	0	0	0	0	0	0	0	0	7	0
Bacillus subtilis	45	0	0	0	0	0	0	7	7	7	0
Staphylococcus aureus	41	0	0	0	0	0	0	0	0	0	0
Sarcina lutea	50	0	0	0	0	0	7	12	14	0	0
Gram-negative bacteria											
Escherichia coli	35	0	7	0	0	0	0	7	0	0	0
Pseudomonas aeruginosa	46	0	0	0	0	0	7	7	0	7	0
Salmonella paratyphi	50	0	0	0	0	0	11	9	12	10	0
Salmonella typhi	44	0	0	0	0	0	0	0	0	0	0
Shigella boydii	40	0	7	0	0	0	0	10	15	10	0
Sh. dysenteriae	50	0	10	0	0	0	8	8	9	11	0
Vibrio mimicus	43	0	0	0	0	0	7	7	7	7	0
Vibrio parahemolyticus	40	0	0	0	0	0	0	0	0	0	0

Abbreviations: AQ, aqueous soluble fraction; CL, chloroform soluble fraction; DCM, dichloromethane soluble fraction; ME, methanolic extract; PE, pet-ether soluble fraction; STD, standard drug ciprofloxacin.

results were presented in Table 4. The statistically significant peripheral analgesic response was obtained in the current investigation ($p < 0.001$). The value of % inhibition of writhing counts found in 200 and 400 mg/kg bw dosages were 60.19 ± 0.49% and 70.37 ± 0.33% in case of leaf extract, and 45.37 ± 0.30% and 50.93 ± 0.87% in bark extract, respectively. However, the standard drug diclofenac sodium showed 80.56 ± 0.22% inhibition.

3.7 | Antidiarrheal activity

The crude extract of leaves and bark of *W. fruticosa*, at the doses of 200 and 400 mg/kg bw were estimated to determine whether they possess antidiarrheal ability or not. The findings of the assay revealed that both doses of bark extract ($p < 0.001$) and only 400 mg/kg bw of leaf extract ($p < 0.05$) were found to possess

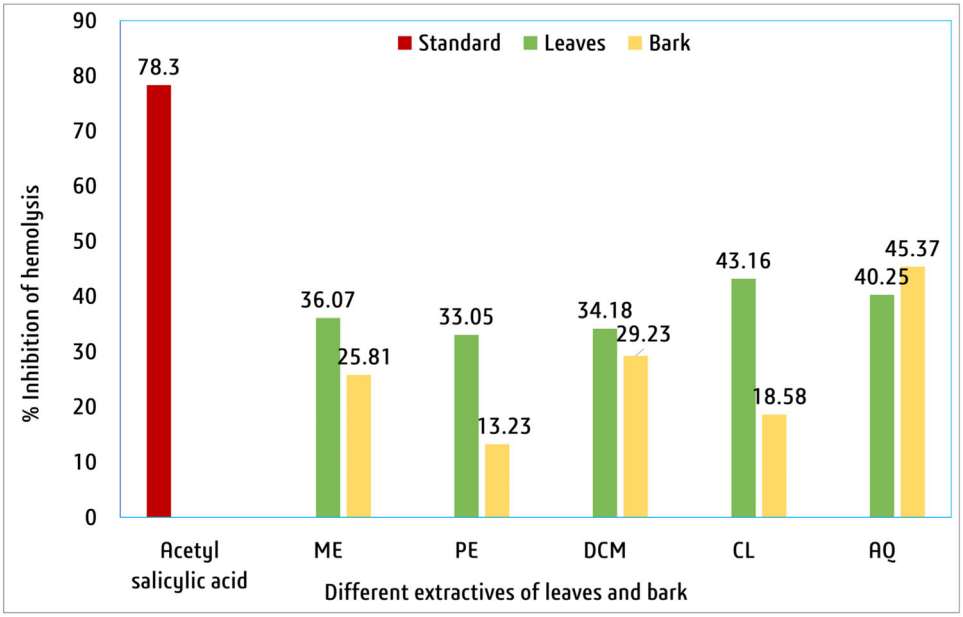

FIGURE 4 Percentage (%) of inhibition of hemolysis of different extractives of leaves and bark of *Woodfordia fruticosa* in hypotonic solution-induced condition. AQ, aqueous soluble fraction; CL, chloroform soluble fraction; DCM, dichloromethane soluble fraction; ME, methanolic extract; PE, pet-ether soluble fraction.

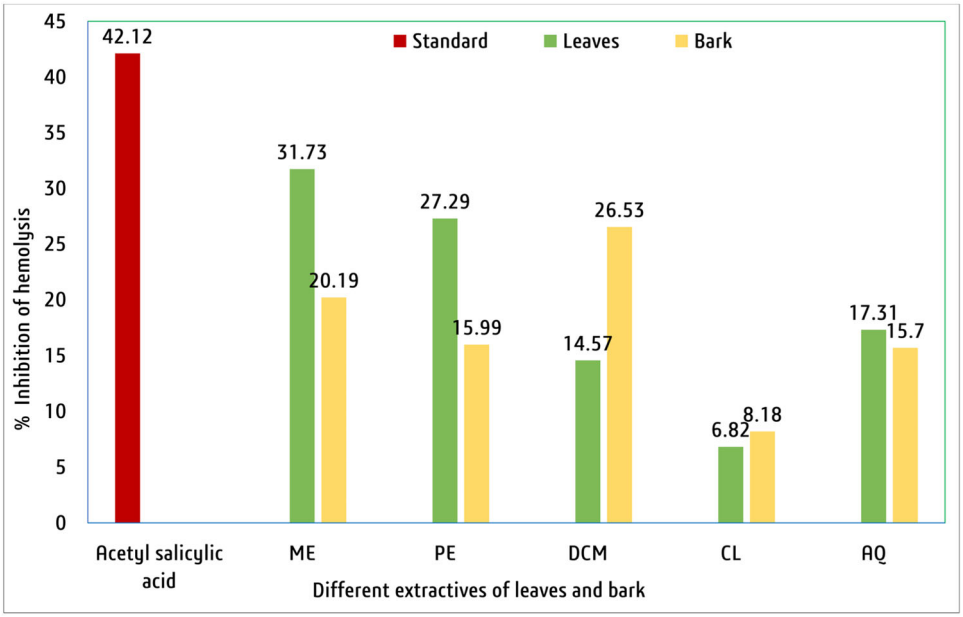

FIGURE 5 Percentage (%) inhibition of hemolysis of different extractives of leaves and bark of *Woodfordia fruticosa* on heat-induced conditions. AQ, aqueous soluble fraction; CL, chloroform soluble fraction; DCM, dichloromethane soluble fraction; ME, methanolic extract; PE, pet-ether soluble fraction.

statistically significant antidiarrheal activities. After 4 h of drug administration, the doses of leaf extracts reduced diarrheal feces of 68.75% and 29.17%, respectively. In the case of bark, reduction activity was found to be significant by reducing diarrheal feces of 56.25% and 62.50% at two doses, respectively, after 4 h of test sample administration (Table 5).

3.8 | Acute oral toxicity

Throughout the 14-day observation period, all mice subjected to varying doses of both *W. fruticosa* extracts (ranging from 1000 to 5000 mg/kg) survived. Since no mice died after taking this dosage, the median lethal dose (LD_{50}) value for both extracts exceeded

TABLE 3 Central analgesic activities of methanol extract of leaf (MEL) and bark (MEB) of *Woodfordia fruticosa* in mice by tail immersion method.

Groups	Treatment	Average time of tail immersion of mice Time after loading the test samples		
		30 min	60 min	90 min
Negative control	Tween 80 solution	2.59 ± 0.15	2.37 ± 0.11	2.34 ± 0.04
Positive control	Morphine 2 mg/kg	5.32 ± 0.07***	10.04 ± 0.06***	13.32 ± 0.39***
I	MEL 200 mg/kg	4.15 ± 0.25***	6.41 ± 0.37***	9.00 ± 0.13***
II	MEL 400 mg/kg	4.87 ± 0.32***	7.03 ± 0.21***	9.23 ± 0.13***
III	MEB 200 mg/kg	3.85 ± 0.14***	6.38 ± 0.09***	7.99 ± 0.34***
IV	MEB 400 mg/kg	4.27 ± 0.17***	7.15 ± 0.10***	9.45 ± 0.07***

Note: Data are represented as mean ± SEM for $n = 6$. ***$p < 0.001$ versus negative control.

TABLE 4 Peripheral analgesic activities of methanol extract of leaf (MEL) and bark (MEB) of *Woodfordia fruticosa* in mice by acetic acid-induced writhing technique.

Groups	Treatment	% Inhibition of writhing
Negative control	Tween 80 solution	-
Positive control	DS 50 mg/kg	80.56 ± 0.22***
I	MEL 200 mg/kg	60.19 ± 0.49***
II	MEL 400 mg/kg	70.37 ± 0.33***
III	MEB 200 mg/kg	45.37 ± 0.30***
IV	MEB 400 mg/kg	50.93 ± 0.87***

Note: Data are presented as mean ± SEM for $n = 6$. ***$p < 0.001$ versus negative control. Abbreviation: DS, diclofenac sodium.

TABLE 5 Antidiarrheal activity of methanolic extract of leaf and bark of *Woodfordia fruticosa* in terms of total number of diarrheal feces (mean ± SEM) and % reduction in diarrheal feces after 4 h of study period.

Groups	Dose	Total number of diarrheal feces after 4 h	% Reduction of diarrhea
CTL	10 mL/kg bw	8.00 ± 0.85	
Loperamide	50 mg/kg bw	2.50 ± 0.61***	68.75
MEL 200	200 mg/kg bw	5.66 ± 0.88	29.17
MEL 400	400 mg/kg bw	4.66 ± 0.66*	41.67
MEB 200	200 mg/kg bw	3.50 ± 0.42***	56.25
MEB 400	400 mg/kg bw	3.00 ± 0.36***	62.50

Note: Data are represented as mean ± SEM for $n = 6$. Abbreviation: MEB, methanol extract of bark; MEL, methanol extract of leaf. ***$p < 0.001$, *$p < 0.05$ versus control.

5000 mg/kg. None of the drugs under investigation caused any noticeable changes in behavior, breathing, skin conditions, bowel motions, discoloration or hair loss, postural abnormalities, or food intake of the mice throughout the study period.

4 | DISCUSSION

Leaf and bark extracts of *W. fruticosa* were screened to observe *in vitro* and *in vivo* pharmacological activities for exploring the undiscovered therapeutic effects of the plant species and justifying its traditional use. Total phenolic content was quantified, as DPPH free radical scavenging activity was reckoned to evaluate the antioxidant potential of the plant. DCM fraction of leaf and ME of bark possess promising amounts of phenolic contents. Hence, these extracts showed free radical scavenging activities congruent with their phenolic contents. Several previous studies revealed that the gallic acid isolated from this plant has strong antioxidant activity comparable to ascorbic acid and gallic acid (Sigma).[40] Phenolic and flavonoid compounds were reported as natural antioxidants since they could quench free radicals and slow down lipid peroxidation.[41] Hence, it might be presumed that the free radical quenching potential of *W. fruticosa* extracts might be attributed to the phenolics and flavonoids.

One of the leading causes of morbidity and mortality across a wide range of vascular diseases is thrombosis. A thrombus or blood clot forms when fibrinogen is converted to fibrin in presence of active thrombin.[42] Damaged tissues discharge a substance called tissue plasminogen activator (t-PA), which converts the plasma protein plasminogen into plasmin, breaking up the blood clot. Fibrinolytic drugs treat people with blocked veins or arteries because they break up thrombi by t-PA and have a thrombolytic impact.[2] Certain cardiac diseases, like atherosclerosis, can be prevented by lowering the accumulation of platelets.[43] Currently, thrombolytic drugs, alteplase, anistreplase, streptokinase, urokinase, and t-PA are widely used for the management of acute myocardial or cerebral infarction and cerebral venous sinus thrombosis to dissolve clots developed in the circulatory system causing vascular blockage.[44] However, these drugs often cause serious side effects, such as bleeding problems, along with re-occlusion and reinfarction. Several herbs have traditionally been used as antithrombotic agents, and some of their blood clot lysis abilities have been proven scientifically.[45] Therefore, attempts are ongoing to discover new thrombolytic agents from plant origin. In the present study, aqueous fraction of leaf and crude

methanol extract of bark of *W. fruticosa* displayed the highest clot lysis properties, compared with thrombolytic drug streptokinase. The other fractions exhibited moderate antithrombotic activities. Further study should be targeted to find active components that would possess potential against stroke, myocardial infarction, etc.

Since ancient years, infectious diseases caused by bacteria have been emerging rapidly, stressing the public health condition worldwide.[46] The threat posed by increasing antimicrobial resistance (AMR) to world health is alarming. Around 700,000 individuals died worldwide in 2015 as a result of antibiotic resistance, and this number rose to 12.7 million in 2019.[47] Furthermore, if adequate steps are not taken to manage AMR, it is predicted that by 2050, the mortality rate associated with AMR will be increased to 10 million annually.[48] Herbal treatments are considered up-and-coming options for addressing current and escalating AMR challenges, offering potential protection against infections. Like antibiotics, herbal antimicrobial agents can annihilate bacteria or inhibit their proliferation.[49] In this context, developing new antibacterial drugs from nature could ameliorate infectious diseases and pave the burden of AMR by inhibiting the growth of bacteria.[50] Accordingly, the leaves and bark of *W. fruticosa* were subjected to antimicrobial screening against several gram-positive and gram-negative bacteria. The results revealed that the bark extracts of *W. fruticosa* possess promising potential to inhibit several bacteria of both strains. In contrast, leaf extract of the species only inhibited some gram-negative bacteria *E. coli*, *S. boydii*, and *S. dysenteriae*. Several previous studies utilizing the essential oil and leaf extract of *W. fruticosa* in different solvents also found remarkable effects against bacterial growth.[18,19,51] Therefore, our study might provide opportunities to study the antimicrobial effect of the plant constituents more extensively and against other microorganisms so that they could be used to develop antibacterial agents.

Inflammatory cells generate histamine, arachidonic acid, cytokines, and prostaglandins and trigger the synthesis of reactive oxygen species (ROS) that stimulate the inflammatory response.[52] Since the erythrocyte membrane mimics the activities of the lysosomal membrane, stabilization of the erythrocyte membrane can help inhibit the secretion of mediators to inflammation.[53] Hence, the assay for the investigation of membrane stabilizing activity of freshly separated RBC was aimed at finding the *in vitro* anti-inflammatory effects of the plant. All the fractions of *W. fruticosa* leaf and bark extract showed positive responses in hypotonic solution and heat-induced hemolysis of RBC. Flavonoids and phenolic derivatives have been observed to possess antioxidant properties; thereby, they can antagonize the ROS produced to trigger inflammatory reactions.[54,55] Since *W. fruticosa* possesses plenty of phenolics and flavonoids, the ability to stabilize the erythrocyte membrane might be associated with the presence of these phytochemicals.[11,56] Thus, the outcome of the membrane stabilizing activity might bring forth the chance to isolate anti-inflammatory agents.

NSAIDs have been widely used throughout the world to cure pain and inflammation. However, these drugs affect the biological system adversely. Gastric mucosa, renal, cardiovascular, hepatic, and hematologic systems are affected, and tolerance and dependence are induced by opiate analgesics.[57] Subsequently, the discovery and development of new analgesic drugs alternative to NSAIDs and opiates are underway worldwide.[58] The study of the efficacy of plants used as analgesics in traditional medicines has received much attention since they might have fewer side effects.[59] Hence, the analgesic activity of the plant species was evaluated in this study centrally and peripherally. The leaf and bark extracts of *W. fruticosa* showed significant analgesic activity employing tail immersion and acetic acid-induced pain sensation in mice. Heat is applied to the tail of mice to induce pain sensation centrally, and in response, opiate, dopamine, serotonin, and descending noradrenergic neurons become stimulated.[60] On the contrary, peripheral pain sensation is induced by injecting acetic acid in mice intraperitoneally, which, in turn, activates the local peritoneal chemosensitive nociceptors, prostaglandins, and lipooxygenases, causing abdominal constriction.[61–63] Therefore, it can be presumed that the extract of *W. fruticosa* may possess secondary metabolites, such as flavonoids, alkaloids, terpenoids, glycosides, and so on, that can effectively minimize pain sensation by inhibiting the synthesis of endogenous substances involving central and peripheral nervous systems.[64]

Diarrhea is contributing to a large extent to pediatric morbidity and mortality, specifically in developing countries. It usually occurs due to an increased frequency of bowel movements, wet stool, along with abdominal pain.[65] To discover potential antidiarrheal activities from the natural source, the leaf and bark of *W. fruticosa* underwent the investigation of antidiarrheal activity in the present study. All the mice were induced diarrhea by feeding with castor oil, which excessively increased the peristaltic movement. Ricinoleic acid, constituting castor oil, is responsible for the secretion of prostaglandins, which in turn, causes diarrhea.[66] The outcome of the current assessment clearly showed that both doses of bark extract significantly reduced diarrheal feces count compared to the control treatment. On the contrary, only 400 mg/kg of leaf extract exhibited significant antidiarrheal activity. It is noted that 200 mg/kg bw did not show a significant effect because the lesser quantity might not possess enough aptitude to lessen diarrhea.[67] The antidiarrheal potentials of the plant might be characterized by the presence of flavonoids, tannins, sterols, triterpenes, and so on, which might have possessed the antagonistic ability by slowing down gastrointestinal motility and emission caused by castor oil via inhibiting the secretion of autacoids and prostaglandins.[7,68–70] These plant extracts rich in flavonoids may be an effective treatment for both acute and chronic diarrhea because of their capacity to reduce intestinal fluid output and slow bowel motions. Additionally, they might protect the intestines from oxidative stress and preserve the mucosal lining's integrity, potentially minimizing the severity of long-term inflammatory damage.[71]

Preclinical studies in developing new drugs must include toxicity assessments in suitable animal models. Due to people's unwavering belief in the efficacy of the herb and extensive traditional/folk usage of plants, toxicological testing of natural phytoconstituents, medicinal plants, or any new medicine for their harmful potential becomes essential.[72] The acute oral toxicity study found that large dosages of *W. fruticosa* leaf and bark extracts up to 5000 mg/kg did not cause toxicity symptoms, and no mice succumbed during the 14-day observation period. Extracts having LD_{50} values larger than 5000 mg/kg are normally regarded as nontoxic

according to the globally harmonized system (GHS) of Classification and Labeling of Chemicals.[73] Moreover, by employing the formula [human equivalent dose (mg/kg) = animal dose (mg/kg) × (K_m factor of animal/K_m factor of human) = 5000 × (3/37)] described by Reagan-Shaw et al.,[74] it is possible to transform drug doses observed in animal experiments into equivalent doses for humans. In the case of mice, an administration exceeding 5000 mg/kg of methanolic extracts is equivalent to a daily consumption exceeding 24.32 g of leaf or bark extract of *W. fruticosa* for a 60-kg adult human. Thus, the research findings reveal that when it comes to acute levels, crude extracts of *W. fruticosa* can be considered safe.

4.1 | Limitations and future research

The major shortcoming of the study is the absence of a phytochemical investigation of both extracts of the species *W. fruticosa*. To address this, future research should consider conducting an extensive analysis of phytochemicals and isolating the lead compounds. Such an endeavor could pave the way for developing new drugs derived from these plant species, utilizing a combination of *in vitro*, *in vivo* and *in silico* approaches. Additionally, it may be worthwhile to explore the synergistic effects of both extracts in forthcoming studies to determine whether the leaves and bark extracts of the plant species can offer improved efficacy compared to the results presented in this article. Moreover, it is important to note that the *in vivo* oral acute toxicity investigation did not enclose any biochemical or histological analysis of the mice; instead, it relied solely on observational findings.

5 | CONCLUSION

Since the development of herbal medicine opens unparalleled opportunities in the treatment of various ailments, the focus on the exercise of the medicinal system by traditional practitioners is escalating day by day. The findings obtained from this investigation revealed that the methanol extract of the leaves and bark of *W. fruticosa*, along with their different partitionates possessed notable antioxidant, antimicrobial, thrombolytic, and anti-inflammatory properties. Besides, the leaf and bark extracts in methanol were also found to reveal significant analgesic (central and peripheral) and antidiarrheal activities in animal models. In addition, the study on acute oral toxicity demonstrated that both extracts were found to be safe, exhibiting an LD_{50} value exceeding 5000 mg/kg. Therefore, the outcome of the present study justified the traditional uses of *W. fruticosa* and might provide useful information with respect to its identification and isolation of potential candidates for further chemical and biological investigation.

AUTHOR CONTRIBUTIONS

Md. Mahfuzur Rahman: Conceptualization; data curation; formal analysis; methodology; resources; software; validation; visualization; writing—original draft. **Mahfuza A. Soma**: Conceptualization; data curation; formal analysis; project administration; resources; software; validation; visualization; writing—original draft. **Nahid Sultana**: Data curation; formal analysis; validation; visualization; writing—review and editing. **Md. Jamal Hossain**: Conceptualization; data curation; methodology; project administration; resources; software; supervision; validation; visualization; writing—original draft; writing—review and editing. **Md. Abu Sufian**: Investigation; methodology; project administration; resources; software; validation; visualization. **M. Oliur Rahman**: Investigation; methodology; project administration; software; validation; visualization; writing—review and editing. **Mohammad A. Rashid**: Investigation; methodology; project administration; resources; validation; visualization; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The article includes all the essential data needed to substantiate the findings. Additional raw data can be obtained by contacting the corresponding author with a reasonable request.

TRANSPARENCY STATEMENT

The lead author Md. Jamal Hossain affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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