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In Vitro Antibacterial and Thrombolytic Activities and In Vivo Analgesic Activity of the Methanolic Extract of *Xanthium strumarium* (Fruits): A Comprehensive Pharmacological Evaluation

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ABSTRACT

Background: *Xanthium strumarium* is one of the most important medicinal plants whose use in the treatment of various infections and inflammation is well documented in traditional medicines. This study was conducted to investigate the in vitro antibacterial and thrombolytic activities and in vivo analgesic activity of the methanolic extract of *Xanthium strumarium*.

Methods: Antibacterial activity of the extract was investigated against selected gram-positive and gram-negative bacteria using broth dilution technique according to the Clinical and Laboratory Standards Institute (CLSI) guideline with ciprofloxacin as the reference drug. The thrombolytic activity of the extract was measured in the in vitro clot lysis test in human blood sample using streptokinase as positive control and distilled water as negative control. Analgesic activity was screened using acetic acid induced writhing model in Swiss albino mice, with diclofenac sodium as the standard drug.

Results: The extract showed considerable antibacterial activity against the tested organisms by inhibiting growth zones measuring 12 - 27 mm, with maximum activity being observed against *Salmonella typhi* (27 mm), followed by *Salmonella Paratyphi* and *Vibrio mimicus* (23 mm each). Growth zones produced by ciprofloxacin ranged from 25 to 42 mm. The extract caused 31.23 ± 1.26% clot lysis, which was lower than that of 87.87 ± 1.64% produced by streptokinase and higher than that of 5.63 ± 0.48% produced by negative control. The analgesic activity study showed that the extract produced 51.11% inhibition, while diclofenac sodium produced 91.11% inhibition.

Conclusion: *Xanthium strumarium* is characterized by appreciable antibacterial, moderate thrombolytic and considerable peripheral analgesic activities. These results confirm its use in traditional medicines and may provide leads for novel pharmaceutical development.

Keywords: Analgesic Activity; Antibacterial Activity; Methanolic Extract; Medicinal Plants; Phytochemicals; Thrombolytic Activity; Writhing Test; *Xanthium strumarium*.

1. Introduction

Herbal drugs have long been used as medicines and still maintain their value in the field of general medicine. The World Health Organization (WHO) estimates that about 80% of the people living in developing nations make use of traditional herbal medicines for the prevention and treatment of diseases. In virtue of their phytochemical richness, medicinal plants have recently drawn attention as promising sources of novel bioactive compounds possessing antibacterial, anti-inflammatory, analgesic, antioxidant, and thrombolytic activities. Thus, pharmacological evaluation of medicinal plants became an essential part of the process of creating new therapeutic agents [1-2].

Antibacterial resistance is regarded as one of the major global public health problems nowadays. Improper use of antibiotics has promoted the emergence of resistant bacteria that cause increased morbidity and mortality and pose a threat to healthcare systems. Antibacterial resistance was defined by the WHO as one of the top ten global health threats of the twenty-first century. Hence, finding new antibacterial drugs from natural sources became extremely important. It has been shown that many secondary metabolites produced by medicinal plants, such as alkaloids,

flavonoids, tannins, phenolic substances, saponins, and terpenoids, are able to inhibit the growth of various pathogenic microorganisms and may be regarded as promising alternatives to traditional antibiotics [3-4].

Thrombosis, including myocardial infarction, ischemic stroke, deep vein thrombosis, and pulmonary embolism, is still considered one of the main causes of death among the population. Thrombolytic treatment allows dissolving the fibrin-rich blood clots and restoring blood flow. However, thrombolytic drugs that are available now, including streptokinase, urokinase, and tissue plasminogen activator, are relatively expensive, may cause bleeding and allergic reactions, and are characterized by the short time of action. Therefore, searching for new thrombolytic agents produced by plants is highly relevant now [5].

One of the major complaints among patients suffering from inflammatory conditions and tissue injuries is pain. NSAIDs and opioids are the current treatments of choice for alleviating pain. However, prolonged use of these medications is associated with numerous adverse effects such as gastrointestinal ulceration, renal impairment, hepatotoxicity, cardiovascular side effects, development of tolerance and addiction. For this reason, the use of plants having analgesic effect is gaining popularity among researchers in the search of safer pain medications [6].

Xanthium strumarium L. (family Asteraceae) or common cocklebur is an annual herb native to Asia, Europe, Africa, and America. The plant has been extensively used by traditional medical systems such as Ayurveda, Traditional Chinese Medicine, and various indigenous healing practices in the treatment of fever, headache, arthritis, skin diseases, sinusitis, inflammation, infections, and painful conditions [7-8]. Various parts of the plant, especially leaves, fruits, seeds and aerial parts have been shown to contain various bioactive compounds such as sesquiterpene lactones (xanthatin and xanthinin), flavonoids, phenolic acids, tannins, alkaloids, glycosides, terpenoids, steroids, and saponins, which contribute to its multiple pharmacological activities [9-10].

In the previous literature, various studies have shown that the plant has multiple biological activities including antioxidant, antibacterial, anti-inflammatory, antidiabetic, hepatoprotective, anticancer, wound healing, and analgesic activities. There are studies reporting the inhibitory activity of the plant extracts against both gram positive and gram-negative bacteria. Some research work has also indicated the involvement of the plant in inhibiting platelet aggregation and fibrin degradation processes, thus showing the thrombolytic activity. Nonetheless, studies examining the antibacterial, thrombolytic, and analgesic activities of the plant simultaneously are not well documented [11].

In view of this, the current study was planned with the objective of investigating the in vitro antibacterial, thrombolytic and in vivo analgesic potentials of the methanolic extract of *Xanthium strumarium*. It is anticipated that the results obtained from the current research will offer scientific evidence for the use of this herb in folk medicine along with the discovery of natural therapeutic agents against microbial, thrombotic and pain-related problems.

1.1. Study Objectives

To perform a thorough analysis of the in vitro antibacterial and thrombolytic activity and the in vivo analgesic activity of the methanolic extract of *Xanthium strumarium*, the following objectives have been set for the current study.

1) To conduct a study on the antibacterial activity of the methanolic extract of *Xanthium strumarium* against certain Gram-positive and Gram-negative bacterial species.

2) To conduct a comparative study between the antibacterial activity of the extract and the standard antibiotic ciprofloxacin.

3) To conduct a study on the thrombolytic activity of the methanolic extract in vitro by clot lysis test method.

4) To conduct a comparative study between the thrombolytic activity of the extract and the standard thrombolytic agent streptokinase.

5) To conduct a study on the in vivo analgesic activity of the methanolic extract using acetic acid induced writhing method in Swiss albino mice.

6) To conduct a comparative study between the analgesic activity of the extract and the standard drug diclofenac sodium.

7) To study the pharmacological potential of the methanolic extract of *Xanthium strumarium* as a natural source of bioactive compounds with antibacterial, thrombolytic, and analgesic activities.

2. Literature Review

Various pharmacological studies have shown that *Xanthium strumarium* is endowed with various biological properties including antibacterial, antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, anticancer, wound healing, and analgesic properties. All these pharmacological properties are due to the wide range of phytochemicals present in *Xanthium strumarium*. They include flavonoids, phenols, sesquiterpene lactones, alkaloids, terpenes, tannins, saponins, and glycosides. These phytochemicals are known to exhibit a wide range of biological activities independently and in combination [2,6].

Numerous studies have confirmed the antibacterial effects of extracts from *Xanthium strumarium* on gram-positive and gram-negative bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella spp.*, and *Bacillus spp*. Antibacterial activity has been attributed to phytochemicals that inhibit bacterial cells by inhibiting the processes of nucleic acid and protein synthesis, and destroying cell membranes. Various extracts of *Xanthium strumarium* have shown varying antibacterial efficacies depending on the polarity of solvents used, parts of the plant, and extraction methods [4].

There are relatively few studies concerning the ability of *Xanthium strumarium* to lyse blood clots. However, existing literature indicates that extracts of *Xanthium strumarium* as well as phytoconstituents show antiplatelet and antithrombotic activities via modulation of platelet activation pathways and reduction of thrombus formation. Experiments have confirmed these biological activities; however, it needs further study to understand the mechanisms of these biological effects to confirm the plant as a source of thrombolytic agents [5].

Analgesic activity of the plant has been proven in various animal models. Studies have shown that methanolic extract is effective in reducing acetic acid-induced writhing response, which means it shows considerable peripheral analgesic activity. This property is linked to the inhibition of the inflammatory mediators, which include

prostaglandins, nitric oxide, tumor necrosis factor alpha (TNF-α), and cyclooxygenase-2 (COX-2). This means that the plant extract works by suppressing inflammation pathways causing pain perception [12-14].

The current studies still prove the potentiality of *Xanthium strumarium* from several research points of view despite several deficiencies that need to be addressed. In spite of the established activity of the plant in terms of being antibacterial, anti-inflammatory, antioxidative, anticancerous and analgesic, there are only few studies proving all of the above properties at once using the same methanolic extract under the same experimental conditions. What is more, the exact phytochemicals behind such properties, the exact mechanisms of the action of these compounds, their pharmacokinetic properties and safety profile are not yet well-studied. So, further systematic pharmacological studies are needed to confirm the potential of *Xanthium strumarium* and give experimental proofs for its medical use.

3. Materials and Methods

3.1. Chemicals and Drugs: Acetic acid, diclofenac sodium, methanol, and ciprofloxacin were purchased from Merck Co., Germany. All reagents were analytical grade.

3.2. Experimental animals: Eight-week-old Swiss albino mice with an average weight of 27 to 30 grams were utilized for this research and were obtained from Jahangirnagar University in Dhaka, Bangladesh. A 12h light and dark cycle and temperatures ranging from 22 to 25°C with 60 to 70% humidity were provided for the animals in cages with appropriate environmental factors [15]. Pellets, used as a maintenance food for mice, sourced from Jahangirnagar University, Dhaka. Animals used in this study strictly followed the regulations of our institute regarding animal use purposes.

3.3. Collection of plants and preparation of extracts: The fruits of the ripened plants of *Xanthium strumarium* belonged to the family Asteraceae; they had been collected from several regions in Noakhali in Bangladesh. Cold extraction or methanol extraction was carried out to clean up the resulting plant parts, the fruits, from any impurities like other plants or plant particles. They were cut into fine particles and left to dry under sunlight for a week. A proper grinders' aid was taken to crush the particles into fine powder. The preparation of the methanolic extract involved soaking a sample of 500 gm dried fruit powder in 1800 ml of methanol in a glass container for a period of 15 days while shaking. After this process, the resulting mixtures were filtered using cotton and then the solution was poured onto the Whatman filter paper [16].

3.4. Antibacterial Activity

Antibacterial property of *Xanthium strumarium* extract was investigated, shown in figure 1(a), against some selected bacterial isolates. These isolates include gram negative bacteria such as *Salmonella typhi*, *Salmonella paratyphi*, *Shigella boydii*, and *Escherichia coli*; and gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus*, and *Vibrio mimicus*. New cultures of bacteria were preserved in isotonic saline solution of 0.9% NaCl and stored in Eppendorf tubes. Bacteria were vortexed to obtain a uniform suspension. After preparation, nutrient agar plates were autoclaved. The cultures of microbe were inoculated on the nutrient agar plates using the aid of cotton swabs. Filter papers cut from sterilized filter papers,

namely Whatman No. 1 with a diameter of 5 mm each were used for the nutrient agar plates. Disks were dipped into 20 µL ethanolic solution of the compound using a micropipette. Incubation was done at 37°C for 24 hours. The zone of inhibition in millimeters was measured in order to assess the antibacterial activity of the compound [17-18].

(a) Antibacterial Test (b) Thrombolytic Test (c) Analgesic Test

Figure 1. Test for pharmacological activity.

3.5. Thrombolytic Activity

Blood was obtained from healthy volunteers (number of volunteers = 5) who had no history of taking anticoagulants or oral contraceptives on the basis of ethical evidence. 1 mL of the blood was placed in pre-weighed sterilized microcentrifuge tubes and incubated for 45 minutes at 37°C to form clot. The clot weight was measured after removing the serum. For preparation of the extract, 100 mg of the plant material was dissolved in 10 mL of distilled water and filtered. Different concentration ranging from 2 to 10 mg/ml was prepared. About 100 µL of extract solution was added to each clot. Enzyme streptokinase and distilled water were used as positive and negative controls, respectively. The tubes were incubated at 37°C for 90 minutes. After that, released fluid, figure 1(b), was removed, and weight was measured again. Percentage clot lysis was determined from the difference in weight of clot before and after treatment [16, 18-19]. The percentage of clot lysis was calculated as:

$$\text{Clot Lysis}(\%)=\frac{\text{Weight of clot after lysis}}{\text{Weight of clot before lysis}}\times 100 \qquad (1)$$

3.6. Analgesic Activity

Writhe test was conducted in acetic acid induced mice models in order to investigate the effect of extract, shown in figure 1(c), on the pain relief potential. Swiss albino mice were dispelled from food at least 2 h before the start of the experiment and separated into a number of 5 groups (where, n=3) weighing 18-22g were used. Group-1 was injected with normal saline (10 mL/kg) as control group, Group-2 received diclofenac sodium drug (10 mg/kg) and the remaining groups-3,4 and 5 were injected with 100, 200 and 300mg/kg of sample extract respectively. After 30min of saline, diclofenac sodium and plant extract injection, the animals were treated with 1% acetic acid. The number of writings was counted after 5min of acetic acid injection for the period of 10 minutes [15]. The percentage writhing inhibition in the experimental and standard samples was compared to the writhing means in the control group using this equation:

$$\text{Writhing Inhibition (\%)} = 1 - \frac{\text{Mean of writhes in sample/standard group}}{\text{Mean of writhes in control group}} \times 100 \quad (2)$$

4. Result and Discussion

Antibacterial, thrombolytic and analgesic potentials of the methanolic extract of *Xanthium strumarium* were investigated in the present study and the findings revealed that the extract contains interesting pharmacological properties and can be considered for further research for therapeutic application as well as its traditional medicinal uses.

4.1. Antibacterial Activity

Antibacterial activity of the methanolic extract is summarized in Table 1 and Figure 2. Methanolic extract showed broad spectrum antibacterial activity against all the tested microbes with inhibition zones between 12 to 27 mm. Highest antibacterial activity of the extract was observed against *Salmonella typhi* (27 mm) and *Salmonella Paratyphi* and *Vibrio mimicus* (23 mm each) while the lowest activity was noted against *Staphylococcus aureus* (12 mm). Naturally, the standard antibiotic ciprofloxacin provided larger inhibition zones (25-42 mm), proving its excellent antibacterial activity. However, methanolic extract showed substantial antibacterial activity against both gram positive and negative bacteria which is an indication of the presence of wide spectrum antibacterial compounds in the extract. The antibacterial activity could be due to some phytochemicals like flavonoids, phenols, tannins, alkaloids, terpenoids, and sesquiterpene lactones, which are known to have membrane disrupting, DNA/RNA synthesis inhibition, enzyme inhibition, and antibacterial growth inhibiting actions. Similar antibacterial activities of the extract of *Xanthium strumarium* have been reported earlier but with variation in antibacterial activities may result due to variations in methods of extraction, origin of plants, phytochemistry of the extract, microbial strains, and method of experimentations [13].

Table 1. Antibacterial activity of the methanolic extract of *Xanthium strumarium* against selected bacterial strains.

Bacteria Class	**Bacteria name**	**Zone of Inhibition (mm)**	
		Xanthium strumarium	Ciprofloxacin (5mcg/disc)
Gram positive bacteria	*Staphylococcus aureus*	12	34
	Bacillus megaterium	18	32
	Bacillus subtilis	20	37
	Vibrio mimicus	23	37
	Bacillus cereus	19	28
Gram negative bacteria	*Salmonella Para typhi*	23	41
	Shigella boydii	22	36
	Salmonella typhi	27	42
	Escherichia coli	20	25

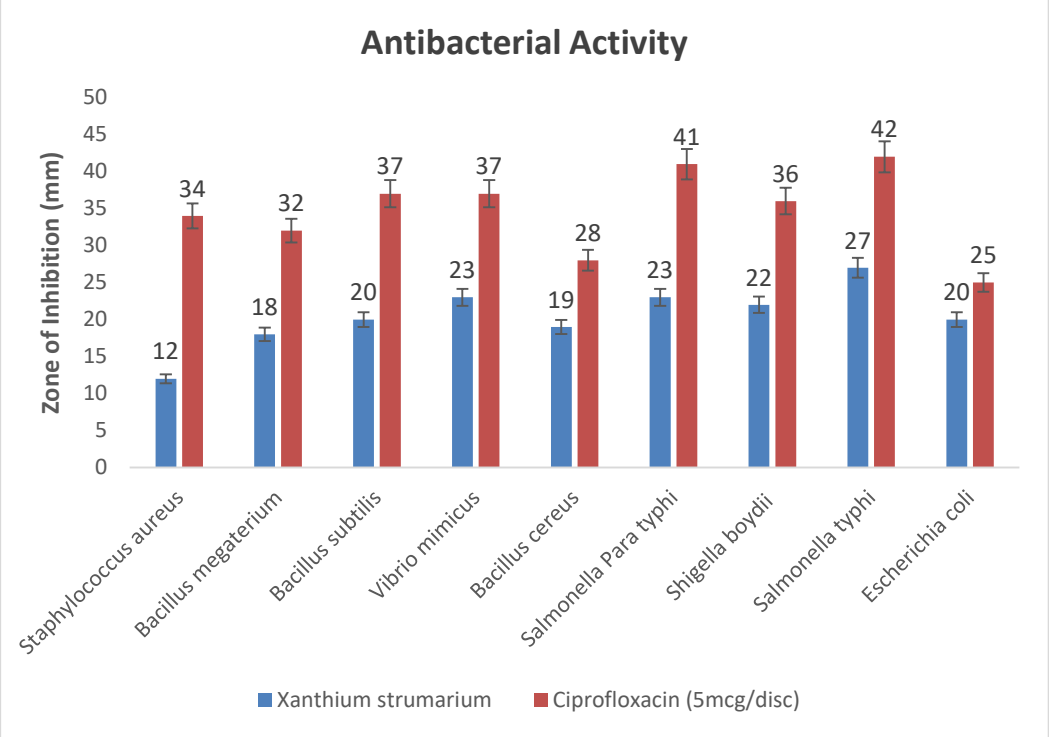

Figure 2. Comparative antibacterial activity of the methanolic extract of *Xanthium strumarium* and ciprofloxacin against selected bacterial strains.

4.2. Thrombolytic Activity

From the results, calculated using equation 1, shown in Table 2 and Figure 3, it was clear that the methanolic extract exhibited 31.23 ± 1.26% clot lysis activity while the positive control gave 87.87 ± 1.64%, and the negative control gave 5.63 ± 0.48%. Although there was low thrombolytic activity in the methanolic extract when compared with streptokinase, the level of clot lysing activity was much higher than in the negative control, hence showing the existence of pharmacologically active compounds that could promote fibrinolysis. Flavonoids, polyphenols, and triterpenoids have been documented to reduce platelet aggregation, inhibit thrombus formation, and also interfere with the processes involved in coagulation and fibrinolytic pathways [14]. Therefore, from the limited number of studies performed on the thrombolytic properties of *Xanthium strumarium*, the current results can add more experimental data that may prove the potentiality of using *Xanthium strumarium* as an antithrombotic agent in the future. Additional studies, however, must be performed on the purified compounds.

Table 2. In vitro thrombolytic activity of the methanolic extract of *Xanthium strumarium* determined by clot lysis assay (n = 3).

Sample	**% of Clot Lysis (Mean ± SD)**
Negative control (Distilled water)	5.63 ± 0.48
Positive control (Streptokinase)	87.87 ± 1.64
Xanthium strumarium extract	31.23 ± 1.26

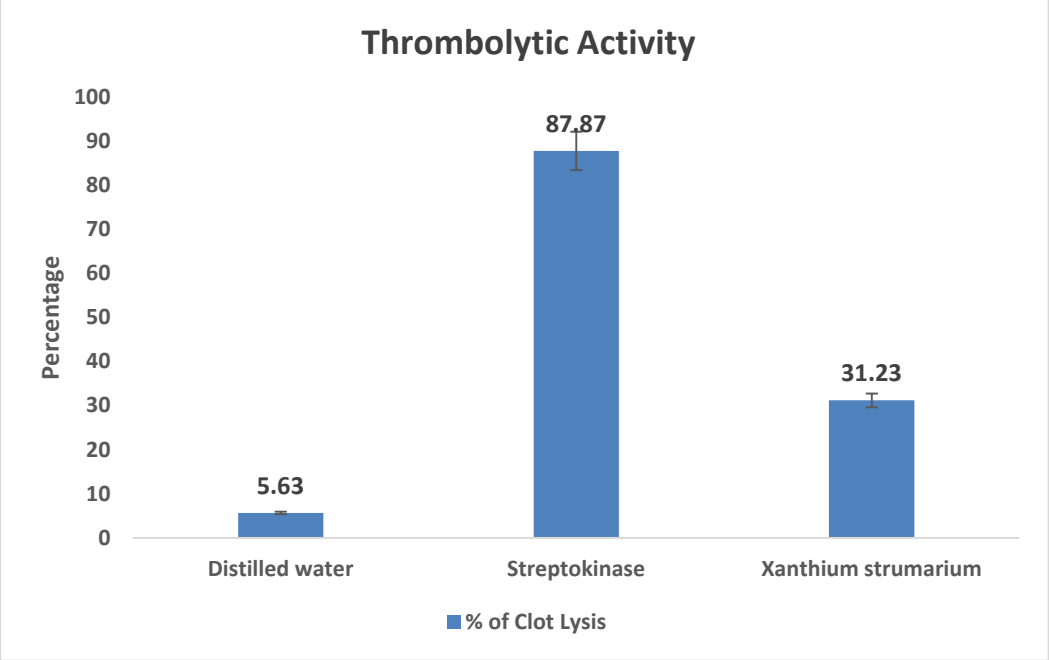

Figure 3. Comparative clot lysis activity of the methanolic extract of *Xanthium strumarium*, streptokinase, and distilled water

4.3. Analgesic Activity

With regard to analgesic activity of the methanolic extract, it was evident from the results, calculated using equation 2, shown in Table 3 and Figure 4 that administration of the extract had a significant analgesic effect on the acetic acid-induced pain in Swiss albino mice as it inhibited writhing by 51.11% compared with 91.11% inhibition of the positive control drug (diclofenac sodium). The acetic acid induced-writhing method is the most commonly used one for testing the peripheral analgesic activity of drugs because acetic acid induces the secretion of several inflammatory mediators like prostaglandins, bradykinin, histamine, and cytokines. From the results obtained, it was evident that the extract suppressed the production of these mediators, hence preventing nociception [6]. Although there was less analgesic activity than diclofenac sodium, the methanolic extract had significant pain inhibiting activity. This concurs with the literature that *Xanthium strumarium* has been found to have significant analgesic and anti-inflammatory activity due to flavonoids, phenolic compounds, and sesquiterpene lactones that inhibit the process of prostaglandin production by COX inhibition.

Table 3. Effect of the methanolic extract of *Xanthium strumarium* on acetic acid-induced writhing in swiss albino mice (n = 5).

Group	Number of writhing (Mean ± SD)	% of writhing inhibition
Control Group	45 ± 1	0
Standard Group (Diclofenac Na 10mg/kg)	4 ± 1	91.11
Xanthium strumarium (500 mg/kg)	22 ± 2	51.11

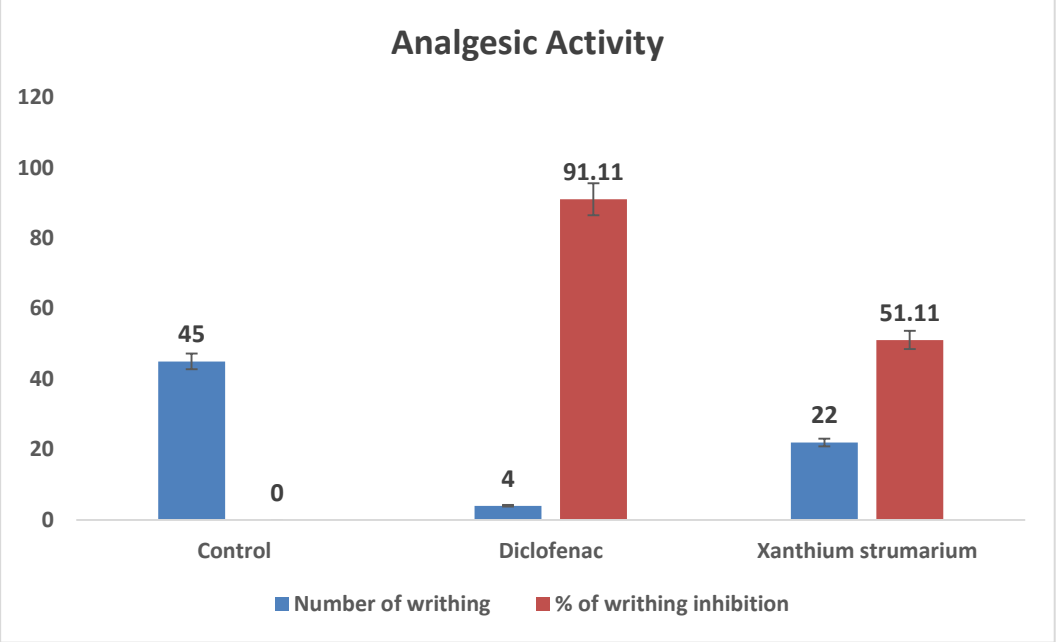

Figure 4. Analgesic activity of the methanolic extract of *Xanthium strumarium* in the acetic acid-induced writhing test.

4.4. Overall Pharmacological Significance

Overall, the outcome of experiments presented in tables 1-3 and figures 2-4 clearly show that methanolic extract of *Xanthium strumarium* is endowed with considerable antibacterial activity, some thrombolytic ability, and noticeable peripheral analgesia. The results offer scientific substantiation for the utilization of the plant in traditional medicine and suggest promising prospects for its further research and development into new medicines.

5. Conclusion and Further Studies

5.1. Conclusion

This current investigation proved the significant antibacterial, thrombolytic, and analgesic properties of the methanolic extract of *Xanthium strumarium*. The extract displayed significant antibacterial activity against gram-positive and gram-negative bacteria, with the greatest inhibitory effect on Salmonella typhi (Table 1, Figure 2). In the thrombolytic test, the extract was found to possess moderate ability to lyse clots (31.23 ± 1.26%) in comparison with streptokinase; therefore, there were active components in the extract which could cause fibrinolytic activity (Table 2, Figure 3). Also, the extract caused a significant inhibition of writhing induced by acetic acid in Swiss albino mice, with 51.11% inhibition rate (Table 3, Figure 4). Overall, the results of this experiment indicate that the methanolic extract of *Xanthium strumarium* contains active components with the possible therapeutic value in treating microbial infections, thrombosis, and pain conditions.

5.2. Further Studies

In order to further validate and expand the results of the current study, the following studies can be performed:

1. Isolation, purification and characterization of the specific bioactive compounds that cause the observed antibacterial, thrombolytic and analgesic properties of the extract.

2. Examination of the molecular and cellular mechanisms of the pharmacological actions of the extract and its components.

3. Studies on various doses to define the optimal dose and pharmacological activity of the extract.

4. Acute, subacute and chronic toxicity studies to define the safety profile of the extract to evaluate it for clinical use.

5. Testing against a wider range of pathogenic microorganisms, including resistant bacteria and fungi.

6. Clinical studies to prove the effectiveness and safety of the *Xanthium strumarium* based drugs in humans.

7. The completion of these studies may assist in developing new herbal therapeutic agents from *Xanthium strumarium*.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare that they have no competing interests related to this work.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' Contribution

Md. Mahbubol Alam, Maksuda Akter Maya and Ahsan Ullah took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing, editing and reviewed the manuscript. Mofizul Islam, Md. Fardin Islam, and Afrin Farzana analyzed data. Fahamida Binta Anower was involved in the design of the manuscript. All authors have read and approved the final manuscript.

Availability of Data and Materials

Supplementary information is available from the authors upon reasonable request.

Institutional Review Board Statement

Not applicable.

Ethical Approval

All animal experiments were conducted in accordance with the institutional guidelines for the care and use of laboratory animals. Every effort was made to ensure the humane handling of animals and to minimize pain, discomfort, and stress throughout the experimental procedures.

Informed Consent

Not applicable for this study.

Acknowledgement

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Declaration of Artificial Intelligence

No AI tools were used for this study.

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