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Evaluation of Antibacterial and Thrombolytic Activities of Ethanolic Leaf Extract of *Stephania japonica*: An in Vitro Study

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ABSTRACT

Stephania japonica is a significant medicinal plant, which has found a wide range of applications in the past and is highly distributed across the nation. The present investigation attempts to look into the antibacterial and thrombolytic activities of ethanolic leaf extract of *Stephania japonica* using an in vitro experiment setup. The samples of the leaves were collected from Chandpur, Chittagong and dried in the shade before grinding into powder. Extraction of the powdered sample was done by means of cold maceration with ethanol as the solvent. The extract's antibacterial activity was assessed against nine species of pathogenic bacteria. Assessment of antimicrobial action was carried out by means of disc diffusion method. Ciprofloxacin was employed as the standard antibacterial compound. Thrombolytic activity was assessed using human blood clots. Streptokinase was employed as the positive control. The extract exhibited strong antimicrobial action against *Salmonella typhi* (17 mm), *Escherichia coli* (16 mm), *Staphylococcus aureus* (13 mm), *Bacillus subtilis* (13 mm), and *Bacillus cereus* (12 mm). The extract didn't display significant activity against some of the tested bacteria. In the thrombolytic activity, the extract showed 30.29% clot lysis, while streptokinase showed 88.49% clot lysis and the negative control showed 5.70%. The results of the study show that *Stephania japonica* possesses moderate antimicrobial and significant thrombolytic potential, and that it is a valuable plant with medicinal properties. The study recommends that the plant is used for medicinal purposes, and that its potential is being realized.

Keywords: Antibacterial Activity; Ciprofloxacin; Disc Diffusion Method; Ethanolic Extract; Medicinal Plant; Pathogenic Bacteria; Phytochemical Potential; *Stephania japonica*; Streptokinase; Thrombolytic Activity.

1.0. Introduction

Medicinal plants are recognized as being of prime importance as a source of therapeutic agents, and medicinal plants are still playing a vital role in the drug discovery process [1]. Traditional herbal medicine provides primary healthcare to a significant portion of the global populace, particularly in developing countries like Bangladesh. Natural products, including plants, are known to have shown various biological activities, and these biological activities are of prime importance in the case of pharmaceutical research [2].

Stephania japonica, a climbing medicinal plant of the Menispermaceae family, is frequently located in tropical and subtropical regions of Asia, including Bangladesh. The plant has been used to cure various diseases such as inflammation, pain, rheumatism, cancer, bone fractures, fever, etc. [3]. The various plant parts, especially the leaves and roots of the plant, have the potential to accumulate various phytoconstituents, making it medicinally important [4]. The availability of the herb for medical uses has prompted the scientific world to investigate its pharmacological significance in regard to *Stephania japonica*.

Thrombotic disorders, such as myocardial infarction, cerebral thrombosis, and pulmonary thromboembolism, have remained major causes of death and disability in the global population [5]. The use of thrombolytics for dissolving the thrombus and resuming blood flow has become common practice; some examples of thrombolytics are

streptokinase and tissue plasminogen activator. Nevertheless, these medicines may also have some side effects; moreover, they are very costly and not readily available. Thus, efforts have been made to identify safe and cost-effective thrombolytics [6].

Besides, the development of drug-resistant microbes is yet another major health issue [7]. In fact, the majority of the pathogenic bacteria have already shown resistance to antibiotics, thereby reducing the efficacy of antibiotics [8]. This above-mentioned scenario clearly depicts the need for new antimicrobial agents with novel mechanisms of action. Medicinal herbs have emerged as viable alternatives to traditional antimicrobials because they contain a variety of secondary compounds that might hinder bacterial development [7].

Even though *Stephania japonica* has been used traditionally for curing infected conditions and inflammatory diseases, the scientific investigation on the thrombolytic and antimicrobial potential of *Stephania japonica* is in its primary stages, especially in the geographical location of Bangladesh. Therefore, the objective of the present investigation was to evaluate the thrombolytic and antimicrobial potential of the ethanol extract of *Stephania japonica* leaves. The result of the present investigation could provide a scientific validation for the traditional use of the plant, as well as the development of new medicinal agents.

1.1. Study Objectives

1) To collect, identify, and prepare the ethanolic leaf extract of *Stephania japonica*.
2) To assess the antibacterial activity of the ethanolic extract against selected Gram-positive and Gram-negative pathogenic bacteria using the disc diffusion method.
3) To determine the zones of inhibition produced by the extract and compare them with the standard antibiotic, ciprofloxacin.
4) To evaluate the thrombolytic potential of the ethanolic extract using an in vitro human blood clot lysis assay.
5) To compare the clot-lysis activity of the extract with the standard thrombolytic agent, streptokinase.
6) To provide scientific evidence supporting the traditional medicinal use of *Stephania japonica* and explore its potential as a source of bioactive compounds for future drug development.

2.0. Literature Review

This herb has been widely used in traditional medicine in order to relieve inflammatory and feverish states, rheumatism, pain, bone fractures, and various infections. The results of previous phytochemical research indicated that *Stephania japonica* contains plenty of different bioactive compounds, such as alkaloids, flavonoids, tannins, phenolics, glycosides, and saponins [2].

Recently, the pharmaceutical importance of *Stephania japonica* has been supported by the results of scientific investigations. Thus, in 2025, Wu et al. conducted comprehensive genomic analysis of this plant and succeeded in assembly of its mitochondrial genome. In this way, they gained important information concerning metabolic pathways and biosynthesis of secondary metabolites that are valuable for pharmaceutics [8].

Das et al. conducted their investigation in 2025, researching the neuropharmacological and memory enhancing activities of methanolic extracts of *Stephania japonica*. The authors found out that they had significant anxiolytic, antidepressant, and cognitive enhancing properties in experimental animals. Molecular docking analysis demonstrated strong interaction between the phytochemicals of the plant and the neurotransmitter receptors; therefore, the results of this study supported the use of the plant for neurological disorders. It was found that these properties were provided mainly due to isoquinoline alkaloids and flavonoids [9].

The antimicrobial properties of the species belonging to the genus of Stephania attracted much attention recently. The results of researches conducted in 2025 demonstrated that the extracts containing a lot of alkaloids and nano-formulations of the plant exhibited high level of antibacterial activity towards both Gram-positive and Gram-negative bacteria. Silver nanoparticles prepared using the extracts from these plants possessed better inhibiting effects; thus, they can be used as antimicrobial agents against the antibiotic-resistant pathogens [10].

Evidence of the pharmacological importance of Stephania species was shown in another investigation conducted by Zhao et al. in 2025. According to the researchers, an extract from the roots of *Stephania japonica* showed antiviral properties concerning porcine epidemic diarrhea virus (PEDV). It was observed that bisbenzylisoquinoline alkaloids from the plant demonstrated good antiviral properties, inhibiting viral entry and replication. The study highlighted the multiple biological activities of *Stephania japonica* and the possibility of obtaining novel therapeutics from it [11].

It was also found that fangchinoline and cepharanthine, which are significant alkaloids belonging to the Stephania group of plants, could inhibit replication of the porcine reproductive and respiratory syndrome virus (PRRSV) by targeting CD163 receptor. The results prove once again the importance of such alkaloids from the therapeutic point of view and their possible application in the development of drugs [12].

In 2025, another research evaluated the wound healing activity of the herbal ointment prepared from the ethanolic extracts of *Stephania japonica*. It was observed that this formulation could accelerate wound contraction and tissue regeneration and was as effective as the standard treatment. This fact shows that phytoconstituents contained in the plant could provide antimicrobial and anti-inflammatory actions involved in the process of wound healing [9].

Phytochemical studies of other species belonging to the Stephania genus have led to the identification of some new bisbenzylisoquinoline alkaloids with antimicrobial, cytotoxic, pesticidal, and anti-inflammatory activities. In 2026, new alkaloids from *Stephania kwangsiensis* were isolated, and their high antibacterial activity against Bacillus cereus was revealed. Thus, the plants of this genus can serve as an excellent source of antimicrobial agents [13].

Although there is a lack of direct evidence regarding the thrombolytic potential of S. japonica, a number of investigations have indicated that the components of medicinal plants, such as flavonoids, alkaloids, and phenolic compounds, increase fibrinolysis, prevent platelet aggregation, and help improve blood vessel function. As the components mentioned above are found in *Stephania japonica*, the potential use of the plant for treatment of circulatory diseases may be related to these properties. This makes research on its thrombolytic activity a reasonable and promising area of scientific exploration [13].

In summary, it can be seen from the review of the literature that the medicinal herb *Stephania japonica* has various biological activities, which include antimicrobial, antiviral, wound healing, neuropharmacological, anti-inflammatory, and even possible thrombolytic properties. Taking into account the wide range of biologically active alkaloids and phenolic compounds found in the plant, the potential of it to provide novel medicines is quite evident.

3.0. Materials and Methods

3.1. Collection and Identification of Plant Material: Leaves of *Stephania japonica* were freshly harvested from the region of Chandpur, Chittagong, Bangladesh. Identification and authentication of the plant material was done by a botanist. Leaves were washed with distilled water to remove dust and foreign substances.

3.2. Preparation of Crude Extract (Cold Extraction Method): The leaves were subsequently dried in the shade at room temperature until a uniform weight was reached. The dried leaves were then crushed to a coarse powder with a mechanical grinder. The leaves, which weighed approximately 430 g, were then soaked in 1200 mL of ethanol in a clean, flat-bottomed container. The container was sealed and left at room temperature for 12 days with sporadic trembling of the leaves being aid in the extraction process. The solution was then filtered through a clean cotton cloth to eliminate contaminants, followed by a Whatman No. 1 filter paper. A clear solution can be obtained using just one filter paper. Evaporation of the solvent was done by a rotary evaporator at decreased pressure to get the dry crude extract, which was stored at 4°C in a container for further use [14-16].

3.3. Antimicrobial Activity Assay

3.3.1. Test Microorganisms: The antibacterial property of the plant extract was evaluated against some selected bacterial isolates that included Gram-negative bacteria like Salmonella typhi, Salmonella paratyphi, Shigella boydii, and Escherichia coli; as well as Gram-positive bacteria like Staphylococcus aureus, Bacillus megaterium, Bacillus subtilis, Bacillus cereus, and Vibrio mimicus [17].

3.3.2. Materials for Antimicrobial Assay: For the antimicrobial assay, various reagents, chemicals, equipment, and laboratory materials were used. The reagents and chemicals included 13.5 g of crude ethanolic extract, 300 mL of ethanol, 9 g of nutrient agar, iodine-free sodium chloride, and 500 mL of distilled water. The glassware used in the experiment consisted of Petri dishes, agar bottles, and glass vials. Essential instruments included a vortex mixer for preparing uniform microbial suspensions and an incubator for maintaining appropriate growth conditions. In addition, non-fragile laboratory materials such as Whatman No. 1 filter paper discs, micropipette tips, and Eppendorf tubes were employed throughout the antimicrobial testing procedure [18].

3.3.3. Preparation of Microbial Suspensions: The newly cultured bacteria were kept in an isotonic saline solution made up of 0.9% NaCl and kept in Eppendorf tubes. Bacteria were vortexed for even suspension before settling to the desired turbidity level [18].

3.3.4. Antimicrobial Assay (Disc Diffusion Method): After preparation, nutrient agar plates were autoclaved. The microbial cultures were inoculated onto the nutrient agar plates by means of the help of cotton swabs. Disks cut out of sterilized filter papers, namely Whatman No. 1, with a diameter of 5 mm each were employed for the

nutrient agar plates. The disks were dipped into an ethanolic solution of 20 µL with a micropipette. The plates were incubated at 37°C for 24 hours. To assess the compound's antibacterial activity, the zone of inhibition was measured in millimeters [19-20].

3.4. Thrombolytic Activity Assay

3.4.1. Preparation of Extract Solution: The 100 mg of the crude extract was dissolved in 10 mL of distilled water and kept overnight. Then the filtrate was taken after filtration through 0.22 µm syringe filter [20].

3.4.2. Preparation of Streptokinase Stock Solution: Five milliliters of sterile distilled water were used to reconstitute commercial lyophilized streptokinase (15,00,000 IU; Popular Pharmaceuticals Ltd.). From this stock, 100 µL (30,000 IU) was used as the positive control for in vitro thrombolytic studies [19].

3.4.3. Clot Formation: Venous blood samples (1 mL) were collected from healthy volunteers who have not received anticoagulant therapy. Ethical approval was granted by the ethical committee of the institution, and all participants gave their consent to participate in the study [20]. Blood samples were collected in pre-weighed sterile microcentrifuge tubes and incubated at 37°C for 45 minutes to allow blood clots to form. Serum was carefully aspirated from the blood clots without disturbing the clots to determine their weights:

$$\text{Clot weight} = \text{Weight of tube with clot} - \text{Weight of empty tube}$$

3.4.4. Thrombolytic Assay Procedure: To each of the tubes containing clots, 100 µL of extract solution was added. The positive and negative controls were given 100 µL of streptokinase and 100 µL of distilled water, respectively. The tubes were then incubated at 37°C for 90 minutes. The fluids were removed after incubation, and the tubes were weighed again to assess the amount of clot lysis [20]. The percentage of clot lysis was calculated as:

$$\text{Clot lysis (\%)} = (\text{Weight of clot after release of fluid} / \text{Clot weight}) \times 100$$

3.5. Statistical Analysis: Each experiment was conducted in triplicate, and the mean ± SD was used to display the results. The data was statistically analyzed using Microsoft Excel software.

4.0. Results and Discussion

4.1. Antimicrobial Activity

The antimicrobial effect is now regarded as an essential therapeutic effect in dealing with the increasing problem of drug-resistant microorganisms [21]. The ethanolic extract of *Stephania japonica* leaves possessed selective antimicrobial activity against both Gram-positive and Gram-negative bacteria. Among the Gram-positive bacteria, Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus exhibited zones of inhibition of 13 mm, 13 mm, and 12 mm, respectively. No activity was recorded for Bacillus megaterium and Vibrio mimicus.

The extract showed moderate effectiveness against Salmonella typhi (17 mm) and Escherichia coli (16 mm) among Gram-negative bacteria, but it was ineffective against Salmonella paratyphi and Shigella boydii. The broad-spectrum efficacy of the common antibiotic ciprofloxacin (5 µg/disc) was confirmed by the wider zones of inhibition it showed against all tested microorganisms. The findings are demonstrated in Table 1 and Figure 1.

Table 1. Antimicrobial Activity of *Stephania japonica* Ethanolic Extract.

Bacteria	Bacteria Name	Zone of Inhibition (mm)	
		Stephania japonica extract	Ciprofloxacin (5µg/disc)
Gram positive bacteria	*Staphylococcus aureus*	13	35
	Bacillus megaterium	0	31
	Bacillus subtilis	13	38
	Vibrio mimicus	0	36
	Bacillus cereus	12	30
Gram negative bacteria	*Salmonella paratyphi*	0	40
	Shigella boydii	0	35
	Salmonella typhi	17	40
	Escherichia coli	16	28

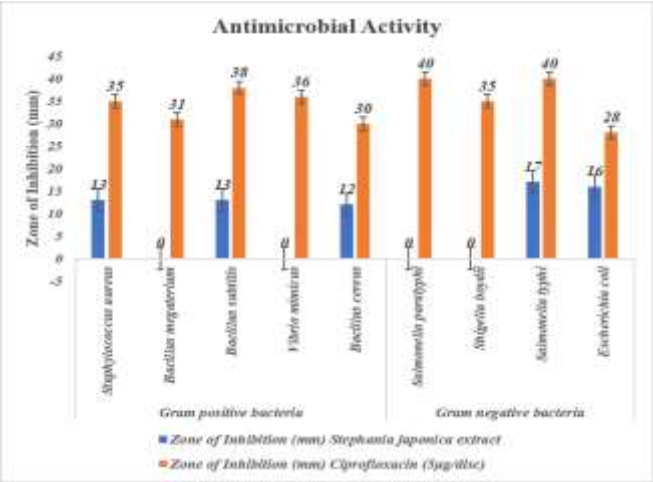

Figure 1. Antimicrobial Activity of *Stephania japonica* Ethanolic Extract.

Based on the above findings, one can conclude that ethanolic extract of *Stephania japonica* is effective in preventing bacterial growth. The strongest bacteriostatic activity has been detected for Salmonella typhi, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus. These results may be due to the presence of certain active compounds like alkaloids, flavonoids, and tannins, which affect the bacterial cell wall structure, preventing protein synthesis.

This is due to the strain specificity of the aforementioned strains, which include Bacillus megaterium, Vibrio mimicus, Shigella boydii, and Salmonella paratyphi, since significant amounts of the extract have to be used in order to inhibit their growth. This corroborates the findings from previous studies on the application of herbs that possess selective antibacterial properties. The study clearly demonstrates the effectiveness of *Stephania japonica* as an antibiotic; however, the efficiency of the herb as an antibacterial is relatively low as compared to antibiotics.

4.2. Thrombolytic Activity

The thrombolytic activity of the ethanolic extract of *Stephania japonica* was assessed in vitro by performing a clot lysis assay. The findings showed that the ethanolic extract had significant thrombolytic activity, as seen by the

30.29% clot lysis it caused, which is shown in Fig. 2 and the findings are demonstrated in Table 2. Conversely, the negative control group treated with distilled water produced very little clot lysis (5.70%), but the positive control group treated with streptokinase (30,000 IU) produced 88.49% clot lysis.

Table 2. Thrombolytic Activity of *Stephania japonica* Extract

Sample	**% of Clot lysis (Mean ± SD)**
Negative control (Distilled water)	5.70 ± 0.578
Positive control (Streptokinase)	88.49 ± 2.403
Stephania japonica extract	30.29 ± 1.003

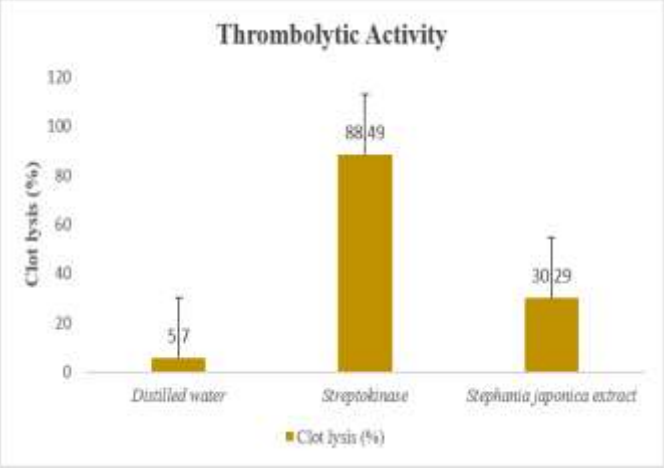

Figure 2. Thrombolytic Activity of *Stephania japonica* Extract

Ethanolic extract demonstrated moderate thrombolytic activity, which could be due to the presence of phytochemicals like flavonoids, alkaloids, and saponins, which are involved in the process of fibrinolysis by activating plasminogen. Even though the percentage of clot lysis in ethanolic extract is less in comparison to streptokinase, its potential in partial clot lysis is significant.

This shows that the negligible lysis observed was indeed because of the plant extract, and it was not because of the degradation of the clots formed spontaneously, as observed in the negative control experiment. This experiment proves the efficacy of the usage of *Stephania japonica* for the treatment of circulation-related disorders, and it has the potential to be used for the production of thrombolytic agents.

5.0. Conclusion and Further Studies

This study's objective was to assess the antibacterial and thrombolytic potentials of the ethanolic extract of *Stephania japonica* leaves. From the results, the ethanolic extract was found to display moderate antibacterial potential against Gram-positive and Gram-negative bacteria, such as Salmonella typhi, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus.

In addition, it was found that the extract possessed a considerable level of thrombolytic activity (30.29% clot lysis) when compared to the negative control, thus confirming its potential use in clot lysis. Although it was found to

possess a lesser level of activity compared to the standard drug used, i.e., streptokinase, it confirms the traditional use of *Stephania japonica* in the treatment of circulatory and inflammatory diseases. Overall, this study revealed that *Stephania japonica* has potential as a natural source for bioactive compounds with antimicrobial and thrombolytic properties, which could potentially be used as a candidate for pharmacological and drug development studies.

Even though these results are encouraging, more research is advised to fully ascertain the therapeutic potential of *Stephania japonica*. The following are recommended for future studies:

1. Phytochemical analysis of the plant extracts must be done in order to identify the bioactive compounds which could have caused the biological activities observed.
2. The isolation and characterization of pure substances by chromatographic and spectroscopic techniques (HPLC, GC/MS, NMR, etc.) should be carried out.
3. Dose-dependent studies are also carried out to ascertain the minimal bactericidal concentrations (MBC) and minimum inhibitory concentrations (MIC) of pathogens.
4. In vivo studies should be conducted to validate the antimicrobial and thrombolytic effects in animal models.
5. Toxicological and safety evaluation is a prerequisite before exploring any potential applications.
6. Molecular mechanism studies could reveal more about the actual pathways for the inhibition of bacteria and lysis of clots.

This would pave the path for the development of new plant-based medicinal medicines in the future. In addition, safer and effective natural drug formulations would be possible.

Declaration

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

Competing Interests Statement

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' Contributions

Fahamida Binta Anower and Md. Mahbubol Alam took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing and editing. Md. Mahbubol Alam, Kazi Nadia Haque Sharna, and Alamgir Hossain analyzed data. Ahsan Ullah, Mst. Munira and Md. Omor Faruk were involved in the design of the manuscript. Ahsan Ullah and Mst. Munira reviewed 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

Not applicable for this study.

Informed Consent

Not applicable for this study.

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Declaration of Artificial Intelligence

Not applicable for this study.

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