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Phytochemical Profiling and Evaluation of Antioxidant, Antimicrobial, and Thrombolytic Activities of Ethanolic Extract of *Tarenna campaniflora* Leaves

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Abstract: Discovery of the genus *Tarenna campaniflora* and its traditional uses was obtained by living in forest or semi-forest areas and by close observations of the indigenous populations. This study evaluated the phytochemical constituents, antioxidant potential, antimicrobial efficacy, and thrombolytic activity of ethanolic leaf extract of *Tarenna campaniflora*. Qualitative phytochemical screening revealed the presence of various bioactive metabolites. DPPH assay demonstrated notable antioxidant activity (IC_{50} = 0.877 µg/mL), comparable to ascorbic acid (IC_{50} = 0.839 µg/mL). Disc diffusion assay showed antimicrobial activity, with the highest inhibition against *Escherichia coli* (18 mm). The extract also exhibited clot lysis (22.42%), compared to streptokinase (31.17%). The findings support the therapeutic potential of *T. campaniflora*, warranting further pharmacological investigations.

Keywords: *Tarenna campaniflora*; phytochemical screening; anti-microbial activity; thrombolytic activity.

1. Introduction

Medicinal plants have been fundamental in traditional medicine, offering a rich source of bioactive compounds with significant therapeutic potential (Khatun et al., 2024). These plants play a vital role in drug discovery, as phytochemical studies help identify the active substances responsible for biological activities. For example, natural products are known to mitigate oxidative stress, a major factor in various diseases (Jones et al., 2017). However, in the past two decades, the reliance on plant metabolites for drug discovery has declined. This is largely due to challenges in using high-throughput screening methods for natural products targeting specific molecular pathways, the complexity of synthesizing natural compounds (Harvey et al., 2015). However, the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is a widely used method to evaluate the antioxidant potential of plant extracts. Research consistently shows a strong correlation between antioxidant activity and the phenolic and flavonoid content of medicinal plants, underscoring their therapeutic potential (Gulcin & Alwasel, 2023).

Oxidative stress arises from an imbalance between free radicals and antioxidants in the body, which plays a critical role in the progression of various diseases, including cancer, cardiovascular conditions, and neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Free radicals, while necessary for certain biological processes, can cause cellular damage when in excess, leading to disrupted cellular functions and structural alterations. Antioxidants help mitigate this damage by neutralizing free radicals, highlighting their importance in maintaining cellular homeostasis and preventing disease progression (Sharifi-Rad et al., 2020; Afzal et al., 2023).

Among medicinal plants, *Tarenna campaniflora*, a member of the Rubiaceae family, has drawn attention due to its diverse traditional uses. Native to tropical and subtropical regions, *T. campaniflora* has been used to treat a range of ailments, including wounds, skin

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infections, gastrointestinal issues, and fever (Rahman et al., 2018; Chowdhury et al., 2019). Its antibacterial properties make it effective against dysentery and diarrhea, while its crushed leaves are applied topically to cuts and minor wounds to promote healing and prevent infections. Preliminary phytochemical screening suggests the presence of secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds, which likely contribute to its therapeutic effects (Yadav et al., 2014; Morguette et al., 2023).

Plants with high antioxidant activity, such as *T. campaniflora*, have garnered interest for their potential to address these issues. Initial findings indicate that *T. campaniflora* contains these bioactive compounds, suggesting its potential as a natural source of antioxidants for managing oxidative stress-related diseases (Jones et al., 2017; Anand et al., 2018).

The global rise in antibiotic resistance underscores the urgent need for new antimicrobial agents. Plants remain a vital source of bioactive compounds with antibacterial and antifungal properties. Studies have highlighted the ability of Rubiaceae species to combat infections (Rahman et al., 2018), making it crucial to assess *T. campaniflora* for its antimicrobial efficacy against clinically significant pathogens. Its historical use in traditional medicine for managing infectious disorders further supports its potential as a valuable resource for addressing the antibiotic resistance crisis (Marina, 2024).

Cardiovascular diseases linked to thrombus formation, such as myocardial infarction and stroke, are rising at an alarming rate. Current thrombolytic agents, including tissue plasminogen activator (t-PA), urokinase (UK), and streptokinase (SK), are associated with risks such as hemorrhage, allergic reactions, and low specificity (Anwar et al., 2016). This has driven efforts to develop safer and more effective alternatives. Natural products with antiplatelet, anticoagulant, antithrombotic, and thrombolytic properties offer significant promise in this regard. Evaluating the thrombolytic activity of *T. campaniflora* could provide insights into its potential applications in managing thrombus-related conditions, addressing existing knowledge gaps, and enhancing our understanding of its pharmacological properties (Bekker et al., 2009; Furie & Furie, 2008).

Finding new sources of medical information is crucial to developing the healthcare sector and enhancing people's quality of life. This study aims to explore the pharmacological potential of *T. campaniflora* through phytochemical screening and by evaluating its DPPH radical scavenging, antimicrobial, and thrombolytic activities. The findings will help validate its traditional uses and highlight its potential for pharmaceutical applications. It will enhance the understanding of the plant's phytopharmacology, supporting its traditional uses and potential pharmaceutical applications.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade chemicals, DPPH from Sigma, USA, $AlCl_3$, NaOH, $Na_2HPO_4 \cdot 2H_2O$, streptokinase, and $NaH_2PO_4 \cdot 2H_2O$ from Loba, India, as well as H_2O_2 from Merck, Germany, and phenazine methosulfate (PMS) from Sigma, USA, were utilized. All required chemicals were acquired from the Department of Pharmacy, Dhaka International University, Bangladesh

2.2. Collection and identification of plant materials

The leaves of *T. campaniflora* used in this experiment were collected from the National Botanical Garden, Mirpur, Dhaka, Bangladesh. The identification of the plant specimen was conducted by Dr. Mohammad Sayedur Rahman, a senior scientific officer at the National Herbarium in Mirpur, Dhaka, Bangladesh, and the authentication number was DACB-7858.

2.3. Preparation of plant extract

To remove moisture, the plant's leaf samples were dried at room temperature. The leaves were then subjected to size reduction to obtain a coarse powder, which was stored in a clean, dry, airtight container. The air-dried leaf 300 gm of *T. campaniflora* powder was dissolved in ethanol for fifteen days. A yellow-brown mass was produced; the extract was then pressure-dried at room temperature in a rotary vacuum evaporator after it was filtered.

2.4. Phytochemical screening

The Phytochemical Screening, as described by Ghani (1998). The chemical constituents of the plant extract were qualitatively identified using standard phytochemical tests, including detection of reducing sugars via color change with Fehling's and Benedict's reagents, alkaloids by precipitate formation with Mayer's and Dragendorff's reagents, saponins through persistent foam formation upon shaking with distilled water, glycosides by color development with sodium hydroxide, steroids through characteristic color changes upon reaction with concentrated sulfuric acid, tannins via blue-black or greenish precipitates with ferric chloride and brown coloration with potassium dichromate, and gums by the appearance of a violet ring with Molisch's reagent (Ghani, 1998). These methods were used to qualitatively examine and classify the chemical components of *T. campaniflora*.

2.5. DPPH radical scavenging activity test

Ten test tubes, each containing specific amounts of plant extract and ascorbic acid at concentrations of: 500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.906, 1.953, and 0.977 µg/mL. To prepare these concentrations, the plant extract and ascorbic acid were measured three times and then dissolved in ethanol. Ascorbic acid acted as the positive control. A precise amount of DPPH was weighed and dissolved in ethanol to prepare a 0.004% (w/v) solution, which was then blended using a Sonicator. Each test tube received 1 mL of the various concentrations of ascorbic acid and plant extract. Subsequently, 3 mL of the 0.004% DPPH solution was pipetted into each test tube. The test tubes were then stored in a dark environment at room temperature for 30 minutes to allow the reaction to occur fully. In addition, blank test tubes containing only ethanol with DPPH were prepared. After this incubation period, the absorbance of each test tube was recorded at 517 nm using a UV spectrophotometer. The percentage of inhibition was determined with the following formula:

Percentage of inhibition = [(*Blank absorbance* - *Sample absorbance*) / *Blank absorbance*] × 100 (Baliyan et al., 2022).

2.6. Determination of antimicrobial activity

To evaluate the extract's antimicrobial activity disc diffusion method was used which was outlined by Fakruddin et al. (2012). Tests were performed on the extract against a range of eight gram-negative microorganisms, which included *Shigella boydii*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Salmonella typhi*, *Vibrio parahaemolyticus*, *Vibrio mimicus*, and *E. coli.* Additionally, there were five gram-positive bacteria tested as well, comprising *Micrococcus luteus*, *Bacillus cereus, Bacillus megaterium*, *Bacillus subtilis*, and *Staphylococcus aureus*. To prepare test plates, Mueller-Hinton agar medium was used. Five-millimeter filter paper discs (Whatman No. 1) were loaded with 25 and 50 micrograms per microliter of crude leaf extract. The discs were completely dry after that. The discs that had been imbued were incubated on sterilized agar plates for 24 hours at a temperature of 37°C, during which they were covered with 100 µL of the culture. For the positive control, discs containing

ciprofloxacin (5 µg) were utilized. After the incubation period, the diameter of the inhibition zone was measured in millimeters. A sterilized blank disc was used as a control reference.

2.7. Thrombolytic activity

This test was conducted in accordance with the procedure outlined by Prasad et al. (2006). Following the addition of 5 mL of sterile distilled water, the commercially available lyophilized streptokinase vial (1,500,000 IU) was thoroughly mixed. An adequate dilution was made from this suspension, which served as the stock solution. To create the clot, 5 ml of venous blood was drawn from 10 healthy volunteers who had never taken oral contraceptives or anticoagulants. Ethical approval and consent were obtained for blood samples. The blood was then divided into 10 sterile micro centrifuge tubes, each 0.5 mL in size, and incubated for 45 minutes at 37 °C. Following this clot formation, the serum was extracted entirely without causing any disruption to the clot, and the weight of the clot was determined by weighing each tube containing the clot once more. Every micro centrifuge tube containing a pre-weighed clot received 100 µL of ethanol extract (10 mg/mL). To the designated control tube, 100 µL of streptokinase was added as a positive control, and 100 µL of distilled water was added as a negative control (Watson et al., 2016). After 90 minutes of incubation at 37 °C, each tube was examined for clot lysis. Following incubation, the discharged fluid was withdrawn, and tubes were weighed once more to determine the weight difference following clot disruption. The percentage of clot lysis was calculated by comparing the weight difference before and after treatment. Based on existing literature, the thrombolytic activity observed may be attributed to the presence of phytochemicals such as flavonoids, tannins, and saponins, which have been reported to exhibit fibrinolytic or anticoagulant effects.

2.8. Statistical analysis

The means ± standard errors of means were used to express all experimental results. One-way analysis of variance was utilized to evaluate statistical significance using Dunnett's test. With Prism 6.0 (Graph Pad Software Inc., San Diego, CA, USA), and Excel, statistical analysis was carried out. When $p < 0.05$, the study's results were deemed statistically significant.

3. Results and discussion

3.1. Phytochemical screening

Phytochemical Screening test revealed that the ethanol extract of *T. campaniflora* leaves contains different types of secondary metabolites.

Plants produce secondary metabolites as part of their natural defense mechanisms. A small number of these secondary chemicals are poisonous to animals, while the others are harmless. Undoubtedly, the majority of such chemicals have been used in human medicine either as entire plants or as unrefined extracts (Hoareau & DaSilva, 1999). Additionally, plants are a natural source of a variety of medicinal properties. The use of these unrefined medications for medical purposes is becoming considerably more common (Joshi et al., 2011). Research has indicated that certain compounds, such as flavonoids, exhibit antioxidant and antidiabetic properties (Katsube et al., 2010). Numerous studies have revealed that different types of polyphenolic compounds, such as tannins, flavonoids, and phenolic acids, have a wide range of biological activities, including strong antioxidant properties (Prakash et al., 2009). *T. campaniflora* is rich in reducing sugar (Fehling's test), Combined reducing sugar, Tannins (Ferric chloride test), Tannins (Potassium dichromate test), Gums, Steroids, Glycoside, Xantho Proteins and Terpenoids; it is low in reducing sugar (Benedict's test), Flavonoids, Saponin and Acidic compounds according to a phytochemical analysis (**Table 1**).

3.2. 1, 1-diphenyl-2-picrylhydrazyl-free radical scavenging activity (DPPH)

The DPPH free radical scavenging assay was conducted to assess the quantitative antioxidant activity, revealing that the IC_{50} values for the ethanol crude extract of *T. campaniflora* were measured at 0.877 µg/mL, while ascorbic acid, the standard reference, had a value of 0.839 µg/mL (**Fig. 1**). *T. campaniflora*, a plant of the *Rubiaceae* family native to Bangladesh, was evaluated for its free radical scavenging ability using the DPPH assay. The extract's effectiveness in neutralizing DPPH free radicals was compared to that of ascorbic acid, a well-known potent antioxidant. The antioxidant activity is a key pharmacological property of medicinal

Table 1. Phytochemical activity of ethanol extract of *Tarenna campaniflora*

Phytochemicals Group	Result
Reducing sugar (Benedict's test)	-
Reducing sugar (Fehling's test)	+
Combined reducing sugar	+
Tannins (Ferric chloride test)	+
Tannins (Potassium dichromate test)	+
Flavonoids	-
Saponin	-
Gums	+
Steroids	+
Alkaloids	+
Glycoside	+
Xantho Proteins	+
Terpenoids	+
Acidic compounds	-

+ = Present, - = Absent

plants. This level of activity aligns with or surpasses that of several other medicinal plants traditionally recognized for their antioxidant potential. For instance, *Ocimum sanctum* (tulsi) and *Azadirachta indica* (neem) have shown IC_{50} values of 2.38 and 3.10 µg/mL, respectively, in similar DPPH assays (Joshi et al., 2011). DPPH is commonly used to evaluate the free radical scavenging or antioxidant potential of plant extracts since it is easily neutralized by antioxidants (Hossain et al., 2019). The ability of the extract to scavenge was found to be dependent on its concentration, represented by IC_{50} (the concentration of the sample needed to reduce the initial concentration of DPPH by 50%). A lower IC_{50} value signifies a greater antioxidant activity. The extract and ascorbic acid's IC_{50} values were determined by linear regression analysis of DPPH percent inhibition vs log concentration plots. The best-fit linear equations were calculated from the experimental data. The IC_{50} was found by replacing 50% inhibition and solving for concentration.

This indicates that the crude extract has strong antioxidant properties compared to the standard. Additionally, the findings imply that the plant contains phytoconstituents that can donate hydrogen, offering protection to cells against potential harm.

3.3. Antimicrobial activity

T. campaniflora showed antimicrobial efficacy against both gram-positive and gram-negative bacteria by utilizing the disk diffusion method (*Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa, and E. coli*) etc. in the disk diffusion assay (**Fig. 2**).

The results revealed that the ethanolic extract of *T. campaniflora* exhibited varying degrees of inhibition at concentrations of 250

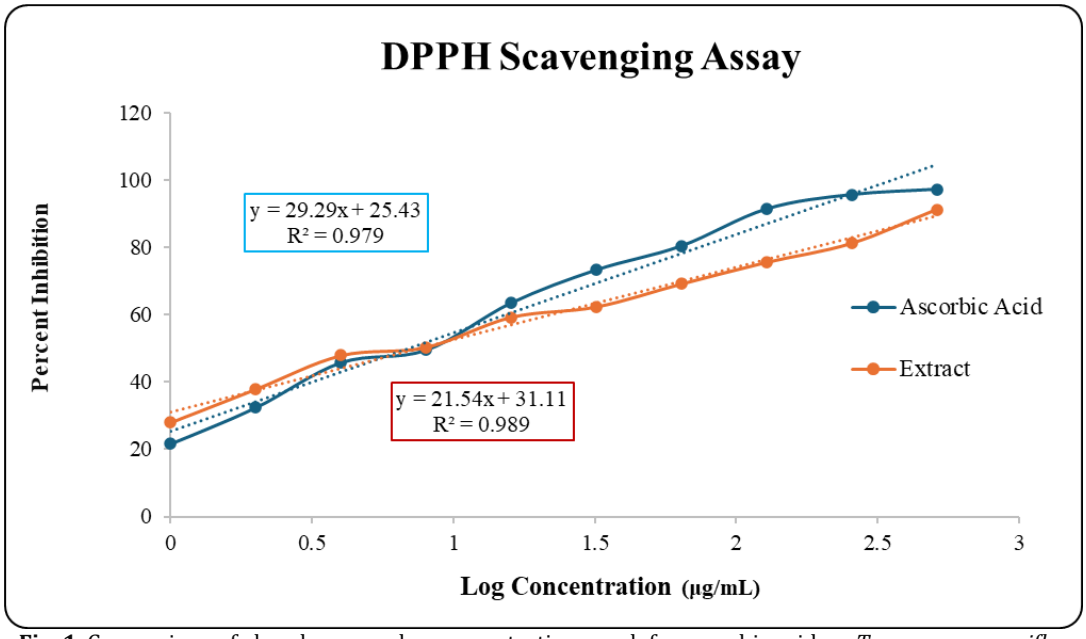

Fig. 1. Comparison of absorbance vs. log concentration graph for ascorbic acid vs. *Tarenna campaniflora*

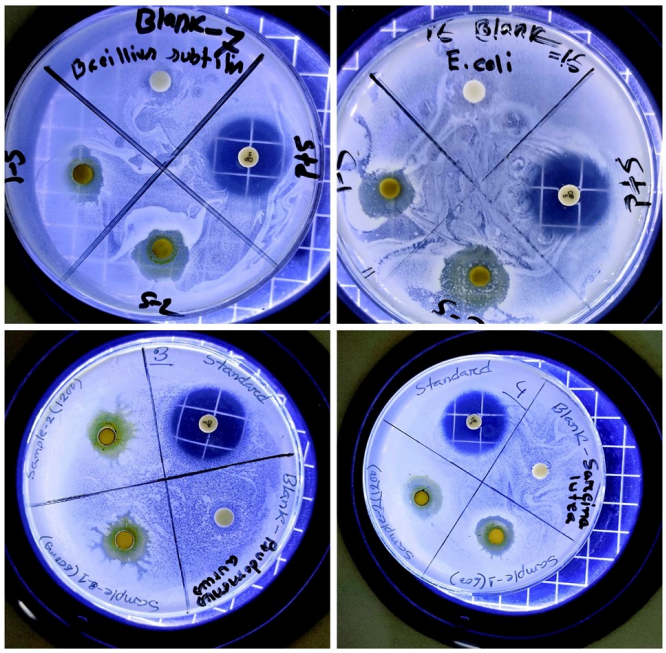

Fig. 2. Microbial zone of inhibition of *Tarenna campaniflora.*

and 500 µg/disc. Notably, *E. coli*, a Gram-negative bacterium, showed the highest sensitivity, with a zone of inhibition measuring 18 mm at 500 µg/disc, which is comparable to inhibition zones observed in *Clausena heptaphylla* and *Morus alba*, both of which have shown 14–20 mm zones of inhibition against *E. coli* in previous studies (Fakruddin et al., 2012; Katsube et al., 2010). In contrast, *Aspergillus niger* exhibited the lowest inhibition, with a zone of only 8 mm at 250 µg/disc (**Fig. 3**).

A standard antibiotic, Ciprofloxacin (5 µg/disc), was used as the positive control. The zone diameters were interpreted according to CLSI (Clinical and Laboratory Standards Institute) M100 guidelines. For example, for *E. coli*, the CLSI interpretation criteria for ciprofloxacin (5 µg) are as follows: susceptible (S) if the zone of inhibition is ≥21 mm, intermediate (I) if it ranges from 16–20 mm, and resistant (R) if it is ≤15 mm. According to this standard, ciprofloxacin demonstrated susceptible activity against *E. coli* with an inhibition zone of approximately 29 mm, whereas the extract exhibited moderate activity with an 18 mm inhibition zone. Although there is no official CLSI standard for plant extracts, comparing them to standard antibiotics provides a good benchmark for assessing the extract's antibacterial potential.

Disc diffusion offers a preliminary indication of antimicrobial activity but does not measure potency. MIC and MBC were not assessed in this study; thus, further quantitative tests are needed to confirm the extract's effectiveness and mode of action.

3.4. Thrombolytic activity

In **Fig. 4**, clot lysis was observed to be 22.42% for the ethanolic extract of *Tarenna campaniflora*, compared to 31.17% for the standard thrombolytic agent, streptokinase. In contrast, the negative control (distilled water) exhibited only 3.05% clot lysis, indicating minimal thrombolytic activity. A statistically significant difference was observed between the extract and the negative control (p = 0.0013), confirming the extract's potential effectiveness in promoting clot lysis. Clot lysis (%) was calculated using the following formula:

% Clot Lysis = (Initial Clot Weight-Clot Weight after Lysis)/Initial Clot Weight ×100%

Generally, thrombin forms blood clots from fibrinogen. Medication classified as thrombolytic or antithrombotic can obstruct the thrombus formation process. Plasmin, which can be activated by activators from inactive plasminogen, is the primary mechanism via which thrombolytic therapy breaks down fibrin (Yuan et al., 2012). Staphylokinase and streptokinase, two cofactor molecules involved in bacterial plasminogen activator, help generate ecosite and improve the enzyme's ability to bind to the substrate. In addition to destroying the extracellular matrix (ECM) and fibrin fibers that keep cells together, staphylokinase stimulates plasminogen to dissolve clots (Ghalloo et al., 2022). When the clots were treated with aqueous and ethanol extracts, a considerable thrombolytic activity was seen when compared to the positive and negative controls. However, the results of the *T. campaniflora* chloroform extract indicate less potential to lyse the clot. The extract from *T. campaniflora* may have thrombolytic activity (Clot lysis) due to the individual compounds or to the combined action of all the active compounds present. Among the tested extracts, the ethanolic extract showed significant thrombolytic activity, whereas the chloroform extract demonstrated considerably less potential to lyse clots. The observed activity in the ethanolic extract could be attributed to one or more bioactive compounds or possibly the synergistic action of multiple constituents present in the extract. Ethical approval and informed consent were obtained prior to collecting human blood samples for this assay, as also noted in the relevant section of the manuscript. The thrombolytic activity of *T. campaniflora* is shown in **Fig. 5.** The possible antioxidant, antimicrobial, and thrombolytic activity mechanism of *T. campaniflora* is shown in **Fig. 4.**

3.5. Novelty of this study

The novelty of this study lies in its comprehensive evaluation of the ethanolic extract of *T. campaniflora*, a plant with traditional medicinal uses but limited scientific exploration. The study successfully highlights the plant's significant antioxidant potential (IC_{50} = 0.877 µg/mL), antimicrobial efficacy against clinically relevant pathogens (notably *E. coli* with an 18 mm inhibition zone), and moderate thrombolytic activity (22.42% clot lysis compared to 31.17% for standard STK). This integrated approach of assessing

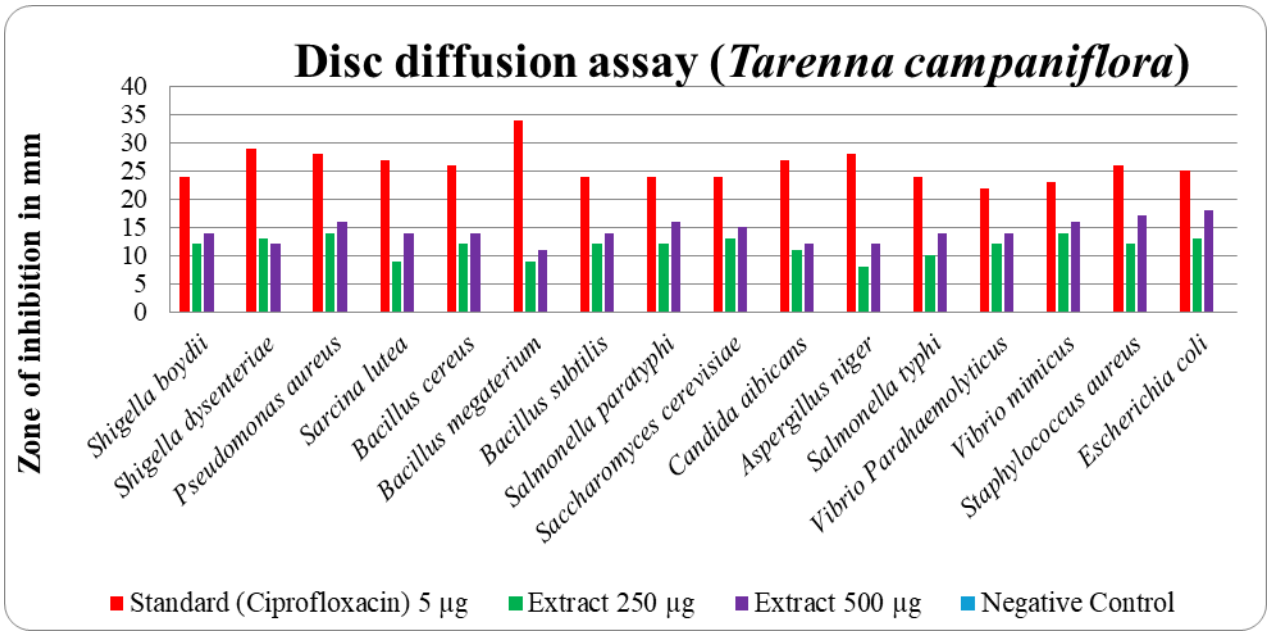

Fig. 3. Antimicrobial activity of ethanolic extract of *Tarenna campaniflora*

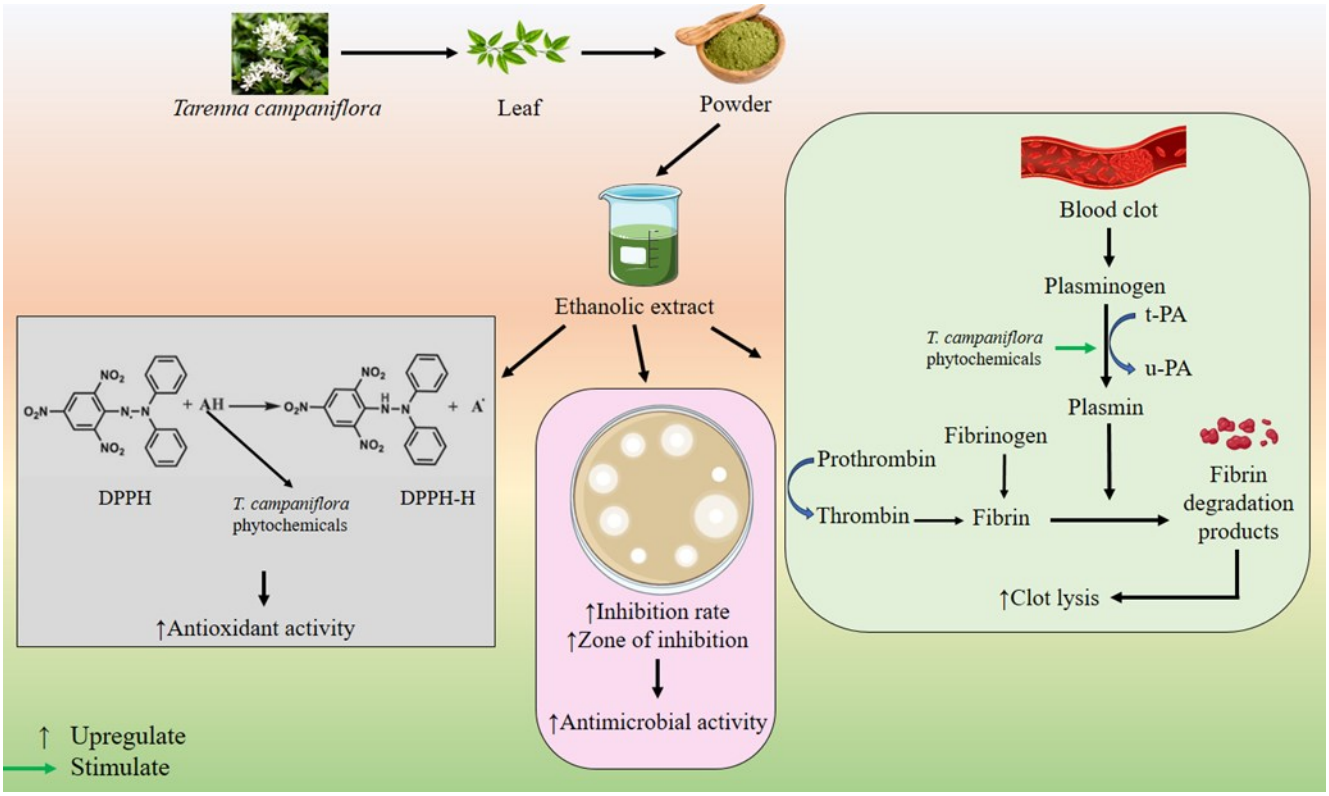

Fig. 4. The possible antioxidant, antimicrobial, and thrombolytic activity mechanism of *Tarenna campaniflora*. [The figure illustrates the proposed pharmacological mechanisms of the ethanolic leaf extract of Tarenna campaniflora. The extract demonstrates antioxidant activity via the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, where phytochemicals donate hydrogen atoms to scavenge DPPH radicals. This converts the purple-colored DPPH radical into its reduced, yellow-colored form (diphenylpicrylhydrazine), resulting in a measurable decrease in absorbance, thereby confirming free radical scavenging activity. The extracts showed antimicrobial activity by an increase in the inhibition rate and zone of inhibition. In the thrombolytic pathway, the phytochemicals are suggested to stimulate the conversion of plasminogen into plasmin via upregulation of t-PA (tissue plasminogen activator) and u-PA (urokinase-type plasminogen activator). Plasmin then breaks down fibrin, which is formed from fibrinogen through the action of thrombin, resulting in fibrin degradation products and enhanced clot lysis. This integrated representation highlights the multifunctional bioactivity of *T. campaniflora* extract and supports its therapeutic potential.]

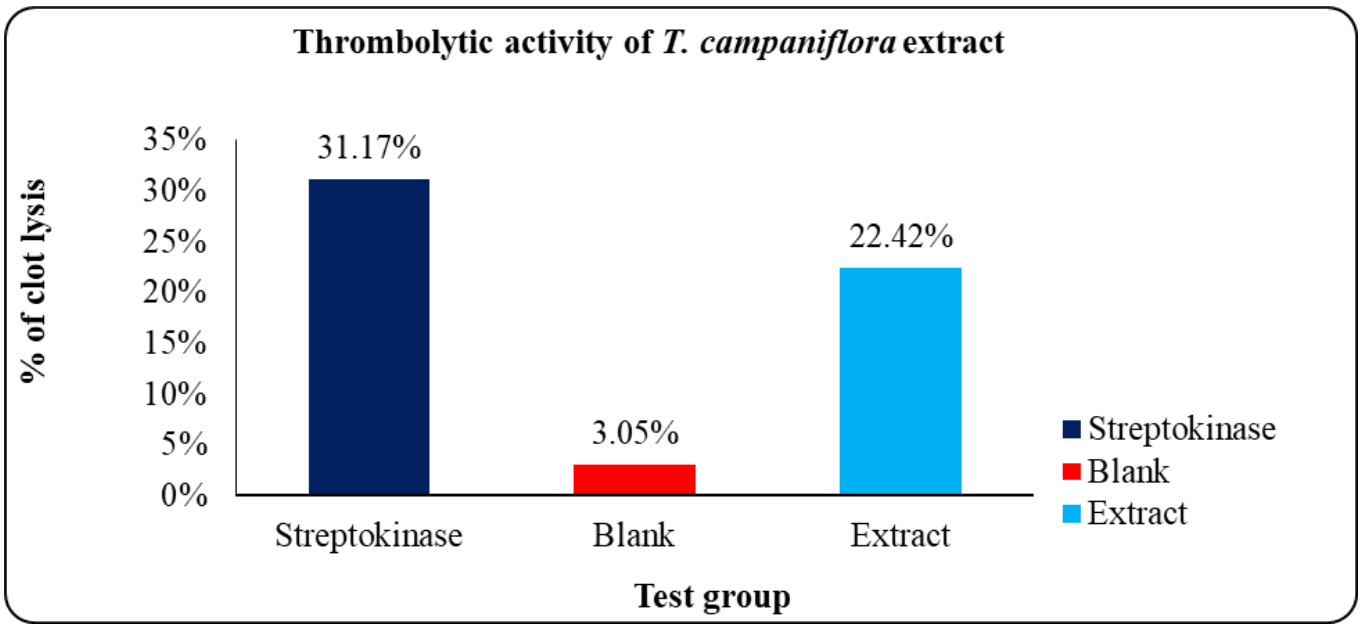

Fig. 5. Thrombolytic activity of ethanol extracts of *Tarenna campaniflora.*

phytochemical constituents, antioxidant, antimicrobial, and thrombolytic properties establishes a foundation for *T. campaniflora*'s pharmacological potential and offers scientific validation for its traditional applications, encouraging further investigation into its bioactive compounds for therapeutic development.

4. Conclusion

The ethanolic extract of *Tarenna campaniflora* demonstrated promising pharmacological potential as evidenced by its phytochemical, antioxidant, antimicrobial, and thrombolytic activities. Phytochemical screening revealed the presence of several bioactive compounds such as reducing sugars (Fehling's test), combined reducing sugars, tannins (ferric chloride and potassium dichromate tests), gums, steroids, glycosides, xantho proteins, and terpenoids. In antioxidant analysis, the extract showed strong free radical scavenging activity with an IC_{50} value of 0.877 µg/mL, which was comparable to the standard ascorbic acid with an IC_{50} of 0.839 µg/mL. Antimicrobial testing using the disc diffusion method indicated that *E. coli*, a gram-negative bacterium, exhibited the highest susceptibility with an 18 mm zone of inhibition at 500 µg per disc, whereas *Aspergillus niger* showed the least inhibition with an 8 mm zone at 250 µg per disc. In the thrombolytic assay, the ethanolic extract achieved 22.42 percent clot lysis, compared to 31.17 percent by the standard streptokinase, while the negative control (distilled water) showed minimal activity at 3.05 percent. These findings support the therapeutic relevance of *Tarenna campaniflora* and validate its traditional medicinal applications, although further investigation is needed to isolate and identify the specific active compounds and understand their mechanisms of action.

Author contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas that is, revising or critically reviewing the article; giving final approval of the version to be published; agreeing on the journal to which the article has been submitted; and confirming to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

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Ethical Approval

This study was approved by the Animal Ethics Committee of Gopalganj Science And Technology University, Gopalganj, Dhaka, Bangladesh (#gstu-19PHR016-005).

Informed consent statement

Not applicable.

Data availability statement

Not applicable.

Acknowledgments

Not applicable.

Conflicts of interest

The authors declare no conflict of interest.

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