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Evaluation of Antioxidant and Thrombolytic Activities of the Methanolic Leaf Extract of *Ageratum conyzoides*: A Comprehensive In *Vitro* Pharmacological Assessment

Lamia Akther Enni[1], Abdul Fahad Sale[1], Fahamida Binta Anower[1,3], Mst. Sharmin Sultana[1], Alamgir Hossain[1], Ohidul Islam[1] & Md. Mahbubol Alam[2,3,*]

[1]*Department of Pharmacy, Faculty of Science, Engineering and Technology, Bangladesh University, Dhaka 1207, Bangladesh.* [2]*Department of Pharmacy, Faculty of Science and Engineering, University of Information Technology and Sciences, Dhaka 1212, Bangladesh.* [3]*Department of Pharmacy, Faculty of Life and Earth Sciences, Jagannath University, Dhaka 1100, Bangladesh. Corresponding Author (Md. Mahbubol Alam) Email: mahbubhasan089@gmail.com*[*]

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ABSTRACT

Background: *Ageratum conyzoides* is one of the medicinal plants used for treatment in traditional medicines and contains several biologically active phytochemical compounds. Therefore, the aim of this study was to analyze the presence of phytochemicals, antioxidant, and thrombolytic activities of the methanol leaf extract of the mentioned plant.

Materials and Methods: Total phenolics (TPC) and flavonoids (TFC) in the plant extract were analyzed using Folin–Ciocalteu and aluminum chloride colorimetry, respectively. Antioxidant activity was measured through DPPH free radical scavenging method. The activity was estimated using ascorbic acid as a positive control. In vitro clot lysis test was performed to estimate thrombolytic activity using streptokinase as positive control.

Results: High levels of phenolic compound content (796.12 mg GAE/g) and flavonoid content (572.88 mg QE/g) were observed in the leaf extracts of the plant. The IC_{50} of methanolic leaf extract was calculated to be 478.18 µg/mL, while that of ascorbic acid was 7.8 µg/mL. Methanolic leaf extract displayed clot lysis percentage as 46.2%, while that of streptokinase and negative control were 88.49% and 5.70%, respectively.

Conclusion: Results obtained in this study suggested that the plant methanolic leaf extract has moderate antioxidant and thrombolytic activities due to presence of phenolic and flavonoids compounds.

Keywords: *Ageratum conyzoides*; Antioxidant Activity; DPPH Assay; In Vitro Pharmacology; Methanolic Leaf Extract; Phytochemicals; Thrombolytic Activity; Total Flavonoid Content; Total Phenolic Content.

1.0. Introduction

Plants have been a good source of medicinal substances since antiquity, providing valuable leads for the synthesis of novel drugs. According to WHO, a considerable portion of the world population uses herbal products as their main health care system. The reason behind the increased interest in herbal medicines lies in their broad spectrum of pharmacological effects, easy accessibility, and relatively low toxicity. Thus, the scientific study of medicinal herbs represents an important topic related to the search for new drugs [1].

Ageratum conyzoides (family Asteraceae) is an annual herbaceous plant distributed throughout the tropical and subtropical areas of Asia, Africa, and South America [2]. It is known to have a broad range of applications in folk medicine as a remedy against wound and burn treatment, fever, inflammation, skin diseases, gastro-intestinal disorders, and infectious diseases [3]. The results of chemical investigation of the studied plant showed that it contains a great variety of bioactive components, such as flavonoids, phenols, alkaloids, terpenoids, tannins, and essential oils [4].

Oxidative stress is a pathologic condition resulting from an unbalance between the levels of produced reactive oxygen species (ROS) and the activity of the antioxidant defense mechanism of a living organism. An excessive ROS generation leads to oxidation, which can cause many illnesses including cardiovascular diseases, diabetes

mellitus, cancer, neurodegenerative diseases, and accelerated aging [5]. Antioxidants prevent cell oxidation by acting as scavengers of ROS and preventing oxidative chain reactions. Phenolic and flavonoid compounds contained in plants are known as very efficient natural antioxidants [6].

Cardiovascular disorders continue to be one of the principal causes of death globally; thrombotic phenomena account for one of the factors that contribute to heart attacks, strokes, and pulmonary embolisms. In spite of the widespread use of various medications, such as streptokinase, urokinase, and tissue plasminogen activator, in the treatment of cardiovascular disorders, their application may have some side effects, such as hemorrhagic events and hypersensitivity reactions. As a result, the development of new drugs for the treatment of thrombosis remains an important challenge to be addressed [7].

Since the biological activities of medicinal herbs are largely determined by their phytochemical characteristics, in particular their phenolic and flavonoid content, the determination of these components is of great significance in assessing the pharmaceutical properties of herbs. In addition, studying the antioxidant and thrombolytic properties of herbal drugs allows establishing the scientific rationale behind their traditional use and the prospect of their further use in the development of medicines [8].

Considering the above, the current research aims to quantify the levels of phenolic and flavonoids in the methanolic extract of the leaves of *Ageratum conyzoides* as well as to assess its antioxidant and thrombolytic properties. The results obtained will contribute to a better comprehension of the pharmaceutical potential of the plant and serve as the background for further research into the development of therapeutic drugs based on medicinal plants.

1.1. Study Objectives

The primary goal of this experiment was to examine the biological properties of the methanol leaf extract of *Ageratum conyzoides*. The particular goals of the study included:

1) Measuring the total phenol content (TPC) of the methanolic leaf extract by applying the Folin-Ciocalteu method.
2) Determination of the total flavonoids content (TFC) of the methanol leaf extract by applying aluminum chloride test.
3) Examination of the in vitro antioxidant activity by carrying out the DPPH free radicals scavenging assay.
4) Assessment of the in vitro thrombolytic activity of the plant extract.
5) Comparing the biological properties of the extract with those of the standard substances.
6) Investigation into the correlation between phytochemicals content and their biological effects.

2.0. Literature Review

Ageratum conyzoides has received significant scientific attention in recent years due to its capacity to synthesize biologically active phytochemicals and therapeutic agents. Phytochemical studies have indicated the presence of different kinds of secondary metabolites in *Ageratum conyzoides*, including flavonoids, phenolic acids, alkaloids, tannins, terpenoids, chromenes, coumarins, and essential oils. All these compounds are responsible for the

pharmacological properties of *Ageratum conyzoides*. Previously, *Ageratum conyzoides* was found to contain quercetin, kaempferol, precocene I, precocene II, and phenolic derivatives with antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties [9-10].

Antioxidant properties of different extracts of *Ageratum conyzoides* were proven in a number of in vitro studies. Chaachouay and Zidane (2024) noted that medicinal plants containing a high concentration of polyphenolic compounds have high antioxidant properties, which can be used in pharmaceutical production [10].

Besides antioxidant activity, *Ageratum conyzoides* exhibits cardiovascular protection. Thrombosis plays an important role in the development of myocardial infarction, stroke, and pulmonary embolism all around the world. Currently available drugs, including streptokinase and tissue plasminogen activator, are highly efficient in treating thrombosis but often cause side effects, such as bleeding and allergy. Thus, the search for new thrombolytic substances based on natural products has become an active field of study [7].

Phenolic compounds found in medicinal plants have been proven to decrease platelet aggregation and improve endothelial function. Recent studies have indicated that flavonoid and polyphenol rich extracts are able to stimulate plasminogen activation pathway, leading to dissolution of clots. Several studies conducted in vitro on medicinal plants proved that phytochemical rich extracts exhibited the ability of clot lysis [11-12].

Recently, advances in phytopharmaceutical research increased the significance of medicinal plants as a source of novel therapeutic agents. Comprehensive review by Chaachouay and Zidane (2024) emphasizes that plant-based natural products still play a crucial role in drug discovery programs [10]. Additionally, recent studies published in 2025 showed that polyphenol-rich botanical extracts have superior antioxidant and cardioprotective activity by several molecular mechanisms like ROS scavenging, anti-inflammatory effects, and regulation of coagulation systems [13]. Zhang et al. in 2025 showed that the medicinal plants rich in phenolics and flavonoids have antioxidant potential and cardioprotective effect through modulation of oxidative stress markers [13].

In 2026, recent studies have been carried out for phytochemical analysis and bioactivity-guided fractionation of medicinal plants. The results of these studies showed a strong correlation between total phenolic content, flavonoid content, and biological activity. It highlighted the significance of phytochemical analysis for medicinal plant studies. Advanced techniques like HPLC, LC-MS, and NMR have become essential for the identification of the active compounds responsible for biological activities [14-17].

Although *Ageratum conyzoides* has been widely used in ethnomedicine and has good biological activities, there is a lack of comprehensive studies regarding its antioxidant and thrombolytic activities. Therefore, the study of antioxidant and thrombolytic activities along with the quantification of phenolic and flavonoid contents would be important for medicinal purpose.

3.0. Materials and Methods

3.1. Chemicals and Drugs: Ascorbic acid, sodium carbonate, quercetin, gallic acid, diclofenac sodium, dimethyl sulfoxide, methanol,1,1-Diphenyl-2-picrylhydrazyl (DPPH) and Folin-Ciocalteu reagent were purchased from Merck Co., Germany. Streptokinase was obtained from Incepta Pharmaceuticals Ltd., Bangladesh.

3.2. Collection of plants and preparation of extracts: *Ageratum conyzoides* plants were collected at Narayangonj in Dhaka, Bangladesh. Cold extraction or methanol extraction was performed to clean the resultant plant parts- the leaves- from unwanted materials, plants or plant fragments. These were cut into small pieces and left to dry in the sun for a week. A suitable grinder was then used to break the fragments down into a coarse powder. It was stored in an airtight container in a dry, cold, and dark place until examination. A clean glass container with a flat bottom was filled with about 430 gm of the powdered substance, which was then steeped in 1200 mL of methanol. The container and the contents were sealed and shook and swirled periodically for ten days. The mixture was then sifted roughly through a clean white cotton towel after 10 days. This was then filtered using Whatman filter paper. For crude extract, the filter was put outside to let the solvent evaporate [18].

3.3. Antioxidant activity

3.3.1. Total flavonoid content: The colorimetric test of aluminum chloride was used for the determination of total flavonoid content [20]. 0.2 mL of the concentration of potassium acetate 1 M, 0.2 mL of aluminum chloride 1% w/v, and 1 mL of the extraction solution 1 milligrams per milliliter were added to 5.4 mL of filtered water and well mixed. Then a blank solution was used to compute the absorbance at 415 nm. The total flavonoid content of the extract was expressed as mg of quercetin equivalent QE/gm of dried extract. For this assay, a calibration curve was created using quercetin standard solutions 0.1–0.5 mg/mL [19-20].

3.3.2. Total phenolic content: The total phenolic content of Ageratum conyzoides was determined by using Folin-Ciocalteu reagent as an oxidizing agent. Standard solutions of gallic acid were prepared in the range from 0.1 to 0.5 mg/mL. Gallic acid 0.5 ml, folin-Ciocalteu reagent 2.5 ml and 2.0 ml of Na_2CO_3 7.5% w/v solution were mixed. Ten milligrams of crude extract, or some of it, were mixed with five milliliters of methanol. This was mixed with 2.5 ml of Folin-Ciocalteu reagent, 2.0 ml of Na_2CO_3 solution and 0.5 ml of plant fractions (2 mg/ml). The mixtures were then left to stand for twenty minutes at room temperature [19-20]. After incubation, absorbance was read at 760 nm by a UV-visible Spectrophotometer (UV-visible Spectrophotometer, Shimadzu, Japan) [2]. The total phenolic content of the test samples was determined from the standard plot of absorbance versus gallic acid concentrations. Milligrams of gallic acid equivalent (GAE) per milligram of extract was used to indicate the sample's phenolic content.

3.3.3. DPPH test for free radical scavenging: The method was used to assess the capacity of the plant fractions for scavenging the stable radicals of 1,1-diphenyl l-2-picrylhydrazyl (DPPH). Ascorbic acid was the standard substance. They were dissolved in methanol to make a solution of 1000 µg/mL. Further serial dilution produced the working values of 0.977-500 µg/mL. Thereafter, the different constituents were made into methanol solutions of equal concentration.

They prepared 5 mg of DPPH in 250 mL of methanol. Later on, 3.0 mL of DPPH solution was mixed with 2.0 mL of methanol solution containing commercial drugs or fractions. The UV spectrophotometer incubated the solutions at room temperature in the dark for 30 minutes. Absorbance at 517 nm was read against a methanol blank solution. % inhibition of the DPPH free radical was calculated from the formula below:

$$\% \text{ inhibition} = [1 - (A_{sample}/A_{blank})] \times 100$$

A blank is the absorbance of a solution that contains all the reagents but not the test samples. It also refers to the absorbance of a solution that contains standards or fractions. Through the graph of percentage of inhibition vs. concentration, the 50% inhibition (IC_{50}) of the DPPH radical was determined [11, 21-22].

3.4. Thrombolytic activity

The following is the procedure used in testing the thrombolytic activity of the plant fractions. Whole blood was drawn from the healthy individuals in 5 mL aliquots and distributed into several sterile pre-weighed vials (1 mL/tube). The whole blood was allowed to clot at 37 °C for a period of forty-five minutes.

After clotting, the resultant serum was removed, and the vials were weighed again to calculate the weight of the clot. The vials were filled with 100 µL of an aqueous solution that contained 2 mg/mL of the plant components. The standard negative non-thrombolytic control contains 30,000 I.U. of streptokinase and 100 milliliters of clean water. Each mixture was incubated at 37 °C for ninety minutes.

After incubation, the vial weights were again checked after removing the clot's released fluid. This is how thrombolytic activity was calculated as the percentage of clot lysis:

$$\% \text{ of thrombolysis} = (B/A) \times 100$$

Where A and B are the weights of clot removed prior to and during the surgery, respectively [11-12].

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. Result and Discussion

4.1. Total flavonoid content: The total flavonoid content (TFC) in the methanolic leaf extract of *Ageratum conyzoides* was determined by aluminum chloride colorimetric method and quantified in terms of quercetin equivalent (QE). Based on the calibration graph of quercetin solution ($y = 0.0009x + 0.0514$, $R^2 = 0.9943$), the percentage of flavonoid content was calculated to be 572.88 mg QE/g dry leaves, which is indicative of fairly good flavonoid biosynthesis in the leaves. The findings are shown in Fig. 1.

The higher flavonoid content shows that the leaves of *Ageratum conyzoides* are an excellent source of phenolic compounds from plants [2]. Flavonoids are well-known antioxidants because of their radical scavenging abilities and reduction of oxidative damage by donating electrons or hydrogen atoms. Hence, the presence of flavonoids in such a high amount may serve as an excellent antioxidant [6, 20-21].

In summary, the methanolic leaf extract of *Ageratum conyzoides* is rich in flavonoid content. This could be responsible for imparting biological significance in the plant extract; however, future investigations are required to determine the individual flavonoid compounds and their biological significance.

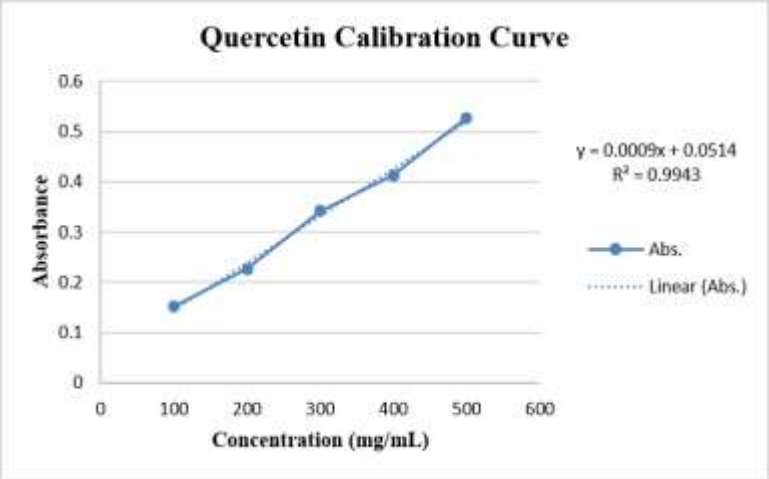

Figure 1. Quercetin standard calibration curve.

4.2. Total phenolic content: The total phenolic content (TPC) in the methanolic leaf extract of *Ageratum conyzoides* was analyzed via the Folin-Ciocalteau method and quantified as milligrams of gallic acid equivalent per gram of dried leaf (mg GAE/g). From the calibration graph of y=0.0008x+0.0587, where R^2 = 0.9271, the phenolic content was found to be 796.12 mg GAE/g, which means there is a significant presence of phenolic components in the extract. The findings are demonstrated in Fig. 2.

It is apparent from the data above that there is an elevated amount of phenolics in the extract. Phenolics are famous for being potent antioxidants because of their ability to donate protons or electrons, thus scavenging free radicals. Hence, it is expected that there will be considerable antioxidant effects due to the measured concentration of phenols in the extract [20-21].

As already mentioned, the total phenolic content turned out to be higher than the content of flavonoids, which is probably due to additional phenolic groups present in the extract [2]. These findings prove the biological activity of *Ageratum conyzoides* leaf extract, confirming the medicinal applications of the species. Nevertheless, there is still a need to conduct a more thorough phytochemical screening in order to determine the nature of bioactive compounds in the extract.

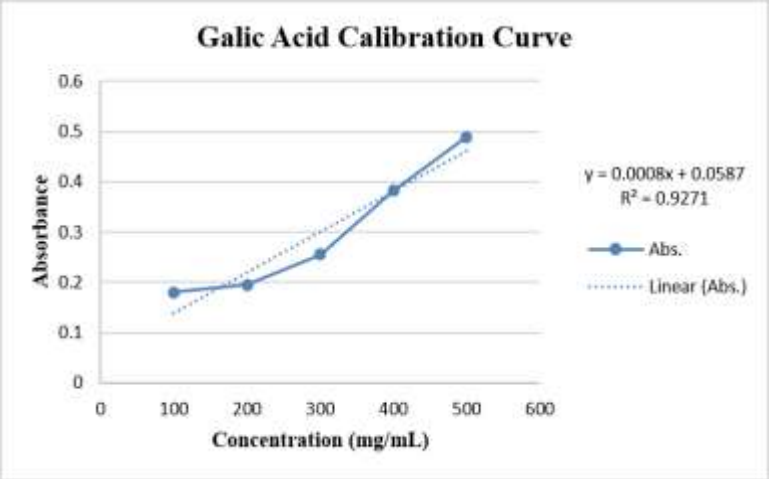

Figure 2. Galic acid standard calibration curve

4.3. DPPH scavenging assay: The antioxidant potential of the methanolic leaf extract of *Ageratum conyzoides* was evaluated using the DPPH free radical scavenging method, where ascorbic acid was used as the standard reference sample, which is shown in Fig. 3. The antioxidant capacity was determined based on the IC_{50} value, whereby the smaller the value, the more potent the radical scavenging property of the substance. Ascorbic acid demonstrated extremely potent antioxidant action with an IC_{50} value of 7.8 µg/mL, while the methanolic leaf extract of *Ageratum conyzoides* possessed an IC_{50} value of 478.18 µg/mL. Thus, the plant extract shows relatively weak activity when compared to the control, though it possesses significant ability to scavenge free radicals.

Antioxidant potential in such cases can be attributed to the high levels of phenolic and flavonoids contained in the extract (796.12 mg GAE/g and 572.88 mg QE/g, respectively), whose antioxidative properties are due to their ability to donate hydrogen atoms or electrons to stabilize free radicals and, therefore, minimize oxidative damage. The IC_{50} value in the case of the extract is probably due to its crude composition, which consists not only of bioactive substances, but also other components with no activity at all, resulting in the decreased efficacy of the substance. Nonetheless, the results prove that the plant leaves show moderate antioxidant activity.

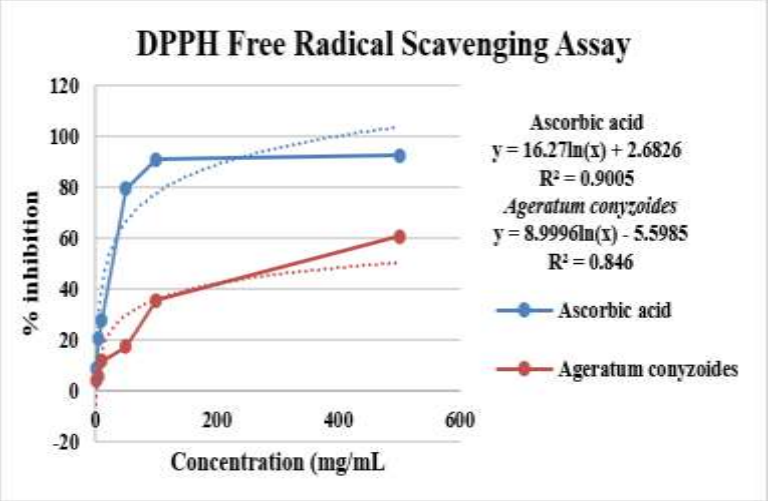

Figure 3. DPPH scavenging activity (% inhibition) of Ascorbic acid and *Ageratum conyzoides* (leaves).

4.4. Thrombolytic Activity: Evaluation of the activity of the methanolic leaf extract of *Ageratum conyzoides* in thrombolysis was carried out through the in vitro clot dissolution assay. The positive control group, Streptokinase, had a clot lysis of 88.49%, while the methanolic extract of *Ageratum conyzoides* had a clot lysis of 46.2%. On the other hand, the negative control group had a clot lysis of 5.70%. The findings are demonstrated in Table 1 and shown in Fig. 4.

Table 1. Thrombolytic activity *Ageratum conyzoides* Extract

Group	**% of Clot lysis (Mean ± SD)**
Standard (Streptokinase)	88.49 ± 0.0465
Control (Distil water)	5.70 ± 3.002
Ageratum conyzoides	46.2 ± 4.781

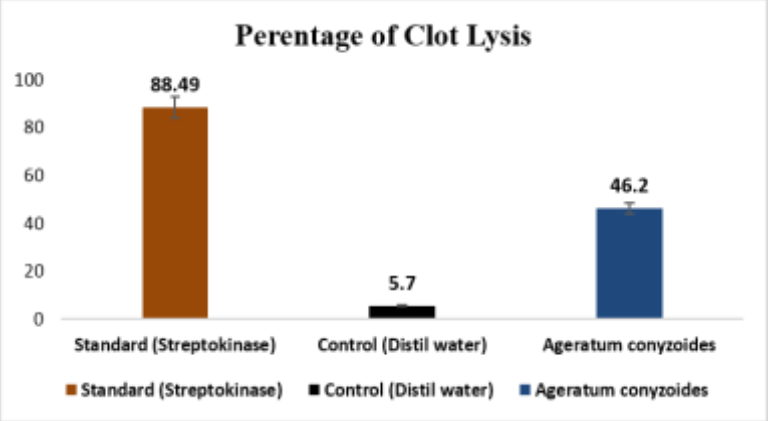

Figure 4. Thrombolytic Activity of *Ageratum conyzoides* Extract

The extract had much higher activity in clot dissolution compared to the control group, showing the presence of biologically active components responsible for thrombolysis. Though the activity is not equal to Streptokinase activity, the extract still has considerable clot dissolution activity. This could be linked to the presence of several phytochemicals in plants such as phenolics and flavonoids, which have a variety of biological activities. Overall, based on the results obtained, it can be said that the methanolic extract of *Ageratum conyzoides* has moderate thrombolytic activity.

5.0. Conclusion and Further Studies

This current study revealed that Ageratum conyzoides leaf extract has high contents of phenolics and flavonoids, 796.12 mg GAE/g and 572.88 mg QE/g of dried leaves, respectively. The leaf extract displayed moderate antioxidant activity based on the result of DPPH IC_{50} of 478.18 µg/mL and appreciable thrombolytic effect based on clot lysis of 46.2%, compared to 88.49% for streptokinase. The biological activities exhibited by this plant might be due to the presence of phenolics and flavonoids in the extract. This study confirms the traditional uses of Ageratum conyzoides in medicine, as well as reveals the potency of this plant in producing antioxidants and thrombolytics.

Despite all these encouraging findings, further investigations are strongly recommended in order to completely determine the potential therapeutic effect of Ageratum conyzoides. The following suggestions are recommended to future researchers:

1. Future research must involve the process of isolation and purification of the phytochemical constituents in Ageratum conyzoides which possess pharmacological activity.
2. Analytical methods such as high-performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LC-MS), gas chromatography mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR) should be used to identify the particular phenolic compound, flavonoids and other bioactive constituents.
3. In vivo studies are necessary to confirm the antioxidant and thrombolytic activities that have been determined in vitro and to evaluate the therapeutic effectiveness of the plant extracts.

4. Acute, subacute and chronic toxicity tests should be done to determine the safety of the compound and the proper dosing regimen for future application.
5. Molecular and cellular mechanisms of the action of antioxidant and thrombolytic effect of the active compounds should be investigated.
6. Further studies are necessary to determine the optimal dose and mode of administration of the compounds to get maximum benefit and minimum side effects.
7. Formulation studies can be done to improve the stability, bioavailability and effectiveness of the bioactive compound.
8. Identification and evaluation of promising bioactive constituents may be considered as potential lead compound in developing phytopharmaceutical products and clinically applicable agents.
9. Synergistic interactions among different phytoconstituents may provide information regarding the increased biological activities of the plant extracts.

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

Lamia Akther Enni and Md. Mahbubol Alam took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing and editing. Abdul Fahad Sale, Fahamida Binta Anower and Md. Mahbubol Alam analyzed data. Ohidul Islam, Mst. Sharmin Sultana and Alamgir Hossain were involved in the design of the manuscript. Md. Mahbubol Alam and Fahamida Binta Anower 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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