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Assessment of Phytochemical Screening, Antimicrobial, Antioxidant, and Thrombolytic Potential of Ethanolic Leaf Extract of *Clerodendrum indicum*

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Abstract: *Clerodendrum indicum* is a traditional medicinal plant known for its therapeutic properties. Its leaves contain various bioactive compounds that may exhibit antimicrobial, antioxidant, and thrombolytic activities. This study aimed to identify major phytochemicals in *C. indicum* leaves and evaluate their antimicrobial, antioxidant, and thrombolytic effects. Ethanolic leaf extract was screened for phytochemicals using standard tests. Antimicrobial activity was assessed via the disc diffusion method against Gram-positive and Gram-negative bacteria. Antioxidant activity was evaluated using the DPPH assay. Thrombolytic activity was tested by measuring clot lysis *in vitro* and comparing it with streptokinase. Phytochemical screening confirmed the presence of alkaloids, tannins, saponins, reducing sugars, steroids, and gums. The extract showed moderate antibacterial activity, particularly against *Salmonella enterica* (27 mm at 500 µg/disc) and *Streptobacillus moniliformis* (24 mm at 500 µg/disc). Antioxidant analysis revealed a dose-dependent scavenging effect with an IC50 of 82 µg/mL. Thrombolytic activity showed 43.87% clot lysis at 20 mg/mL concentration, compared to 69.37% with streptokinase. *C. indicum* leaves possess promising bioactive properties, supporting their traditional use. The extract demonstrated moderate antimicrobial and thrombolytic effects and notable antioxidant potential. Further studies are needed to isolate specific compounds and understand their mechanisms.

Keywords: *Clerodendrum indicum*, Phytochemical screening, Ethanolic extract, Antimicrobial activity, Antioxidant activity, Thrombolytic activity, DPPH assay

1. Introduction

Phytochemicals are naturally occurring, non-nutritive compounds that plants produce to protect themselves from environmental threats (Almodaifer et al., 2017). Although phytochemicals are not essential like vitamins or minerals, research has shown that they may have protective or disease-preventive properties (Chen et al., 2007; Prakash et al., 2012). The major categories of phytochemicals include fibers, glucosinolates, alkaloids, polyphenols, flavonoids, iso -flavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, and phytosterols (Sharma et al., 2011). Herbs, grains, fruits, vegetables, and other plant-based foods all contain these bioactive compounds (Craig et al., 1997). According to recent studies, they offer antibacterial, anti-inflammatory, and antioxidant properties, among other health advantages (Sharma et al., 2011).

Phytochemicals, such as alkaloids, phenols, and terpenes, have shown inhibitory effects against drug-resistant pathogens by focusing on bacterial cell communication, efflux pumps, and biofilms (Khare et al., 2021; Álvarez-Mart 2020). Like microorganisms and animals provided Natural products the plants provide natural products that are safe and efficient substitutes for synthetic drugs (Bahadur et al., 2025).

When the body loses the capacity to detoxify reactive oxygen species (ROS), a biological condition arises known as oxidative stress (Sies et al., 1997; Tan et al., 2018). ROS can cause cellular dysfunction by damaging DNA, lipids, and proteins (Wang et al., 2020; Kunwar et al., 2011; Gilgun-Sherki et al., 2001). Several diseases, such as cardiovascular diseases (Wang et al., 2020),

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neurodegenerative diseases like Alzheimer's and Parkinson's diseases (Houldsworth et al., 2024), chronic inflammatory conditions (Tan et al., 2018; Hussain et al., 2016), and age-related diseases (Houldsworth et al., 2024), can be driven by oxidative stress, which can cause cellular and tissue damage. Antioxidants can help to prevent or reduce oxidative damage, which plays a role in protecting against the development and progression of various diseases (Kunwar et al., 2011; Gilgun-Sherki et al., 2001; Houldsworth et al., 2024; Chaudhary et al., 2023). They work by inhibiting free radicals from propagating, prevent them from forming, and strengthen the body's natural defense system (Chaudhary et al., 2023).

Thrombotic disorders are caused by blood clots that develop in veins or arteries, which disrupt normal blood flow and can lead to potentially fatal conditions (Islam et al., 2016; Emran et al., 2015). Stroke, deep vein thrombosis (DVT), myocardial infarction, pulmonary embolism (PE), and peripheral occlusive diseases stand among the most severe consequences, leading causes of mortality worldwide (Emran et al., 2015; Diwan et al., 2021). Thrombolytic agents are frequently used in the medical treatment of thrombosis to break up blood clots and restore normal circulation. To treat these conditions, thrombolytic agents that comprise tissue plasminogen activator (t-PA), urokinase (UK), streptokinase (SK), etc., have been used globally (Emran et al., 2015). Despite their effectiveness, the substantial risk of bleeding associated with current thrombolytic therapy limits their usage and indicates the need for safer alternatives (Islam et al., 2016; Diwan et al., 2021; Mazumder et al., 2022).

Clerodendrum indicum belongs to the Verbenaceae family (Kar et al., 2014; Siddik et al., 2021). It is a fast-growing, tiny tree or deciduous shrub, usually reaching a height of two to three meters (Sushma et al., 2021; Sidde et al., 2018). It features lanceolate to ovate leaves with smooth or slightly toothed edges on long, thin branches (Sidde et al., 2018). The plant produces fragrant, tubular flowers in long clusters with white to pale purple petals. A tiny, dark-colored drupe is its fruit (Sidde et al., 2018). Throughout tropical and subtropical climates, especially in Southeast Asia, *C. indicum* is widely dispersed. It belongs to the broader genus Clerodendrum, which has more than 500 species that are found around the world in warm temperate and tropical climates (Kar et al., 2014). Its leaves, roots, and petals have long been used to treat respiratory disorders, fever, inflammation, and skin conditions (Siddik et al., 2021; Sushma et al., 2021). Because of its anti-allergic properties, it is also used in Sri Lanka to treat allergic rhinitis and asthma (Madhuranga et al., 2023).

This study aimed to identify major phytochemicals in *C. indicum* leaves and evaluate their antimicrobial, antioxidant, and thrombolytic effects.

2.1. Chemicals and Reagents

The chemicals and reagents used in this investigation were 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, and streptokinase (SK). Agar powder was obtained from E. Merck (India) Limited, Worli, Mumbai, and the nutrient agar medium was sourced from Mast Diagnostics, Mast Group Ltd., Merseyside, U.K. E. Merck (India) Limited, Worli, Mumbai, provided the agar powder, while Mast Diagnostics, Mast Group Ltd., Merseyside, U.K., provided the nutritional agar medium. Other substances used were methanol, 80% ethanol, and standard antibiotic discs, including kanamycin and ciprofloxacin. Additionally, soy broth was purchased from DIFCO Laboratories in the United States. Numerous reagents, including Mayer's reagent, Dragendroff's reagent, Fehling's solutions A and B, Benedict's reagent, and Molisch's reagent, were also used for phytochemical screening.

2.2. Collection and Identification of Plant Materials

The plant leaves were collected from the medicinal plant of the Botanical Garden, Mirpur, Dhaka, Bangladesh, at daytime. The plant leaves were identified and authenticated by Tanmoy Dey (ID: 541), Research Officer, Plantation trial unit division, Bangladesh Forest Research Institute, Dhaka, Bangladesh.

2.3. Preparation of Plant Extracts

At first, the collected plant leaves were cut into small pieces and sun-dried for one week. Then the dried leaves were ground into a coarse powder with the help of a suitable blender. The powder was stored in an airtight container in a cool, dark, and dry place until further analysis was commenced. About 183.2 g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 500 mL of ethanol. The container with its contents was sealed and kept for 9 days, accompanied by occasional shaking and stirring. The whole mixture then underwent a coarse filtration by using a vacuum pump. Then it was filtered using a cotton filter. Then the active constituents with ethanol were dried with a rotary evaporator, and this provided a gummy concentrate of reddish black color crude ethanol extract.

2.4. Phytochemical Screening

Alkaloids, glycosides, steroids, gums, reducing sugar, tannins, flavonoids and saponins were identified by preliminary phytochemical tests. A 10 % (w/v) solution of extract in methanol was taken for each test.

2.4.1. Mayer's Test for Alkaloids

Mayer's reagent (freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in 100 mL distilled water) was added to a 2 mL solution of the extract. The formation of yellowish buff colored precipitate indicated the presence of alkaloids (Rahman et al., 2018).

2.4.2. Dragendorff's Test for Alkaloids

A 2 mL solution of the extract was acidified with 0.2 mL of diluted hydrochloric acid and then 1 mL of Dragendroff's reagent (a solution of basic bismuth nitrate, tartaric acid, and potassium iodide) was added. Orange-brown precipitate indicated the presence of alkaloids (Priyank et al., 2011).

2.4.3. Bromine Water Test for Glycosides

In this test, a few drops of bromine water were added to 1 mL of extract solution, no yellow precipitate was produced, which confirmed the absence of glycosides (De et al., 2010).

2.4.4. Fehling's Test for Glycosides

In this test, a small amount of an alcoholic extract of the plant material was taken in water and alcohol and boiled with Fehling's solution. No brick-red precipitate was formed, which considered as an indication for the absence of glycosides (Shubham et al., 2019).

2.4.5. Sulfuric Acid Test for Steroids

Upon taking a 1 mL solution of chloroform extract, 1 mL of sulfuric acid was added. Steroids are indicated by the formation of red color (Evans et al., 2002).

2.4.6. Molisch's Test for Gums

A few drops of molisch's reagent (α-naphthol 10% (w/v) in 90% ethanol) and sulfuric acid were added to 5 mL solution of the extract. Red violet ring produced at the junction of two liquids indicated the presence of gums and carbohydrates (Sofowora et al., 1993).

2.4.7. Benedict's Test for Reducing Sugar

5mL of benedict's solution (blue solution containing $CuSO_4$, sodium carbonate, and sodium citrate) was added to 0.5 mL of aqueous extract of the plant material and heated the mixture in a boiling water bath for 5 minutes. A red color precipitate of cuprous oxide was formed in the presence of reducing sugar (Lokesh et al., 2020).

2.4.8. Fehling's Test for Reducing Sugar

A mixture of equal volumes of Fehling's solutions A and B were added to 2 mL of an aqueous extract and boiled for few minutes. Formation of brick-red precipitate confirmed the presence of reducing sugars (Harborne et al., 1998).

2.4.9. Alpha Naphthol Solution Test for Reducing Sugar

For this test, in a 5 mL extract solution, 2 drops of 5% alpha-naphthol solution (freshly prepared) and 1 mL of sulfuric acid were added on the sides of the test tube. Violet colored ring was formed at the junction of two liquids in the presence of reducing sugars (Devor et al., 1950).

2.4.10. $FeCl_3$ Test for Tannins

For this screening, 1 mL of 5% $FeCl_3$ solution was added to 5 mL of the extract. A greenish-black precipitate was formed and indicated the presence of tannins (Rahman et al., 2018 & Priyank et al., 2011).

2.4.11. Alkaline Reagent Test for Flavonoids

A few drops of sodium hydroxide were added to a small amount of an alcoholic extract of the plant material. No development of an intense yellow color indicates the absence of flavonoids (Priyank et al., 2011).

2.4.12. Frothing Test for Saponins

1 mL of the stock solution of the extract was diluted with 20 mL of distilled water and shaken for 15 minutes. One centimeter layer of foam on the top of the test tube confirmed the presence of saponins (Rahman et al., 2018).

2.5. *In Vitro* Antimicrobial Activity

2.5.1. Disc Diffusion Method

Antimicrobial activity of the crude extract of *C. indicum* was determined by the disc diffusion method (Parekh et al., 2007; Abbes et al., 2014). Both Gram-positive and Gram-negative bacteria were tested (Gram-negative: *Streptobacillus moniliformis*, *Salmonella enterica*, Gram-positive: *Streptococcus pneumoniae*). At first, 2gm agar powder and 3 gm tryptophan soya broth were measured, and 100 mL of distilled water was added and mixed properly. Then the mixture was placed into the autoclave at 121°C for 40-45 min for heat sterilization. Prior to sensitivity testing, each of the bacterial strains was cultured on a nutrient agar plate. Bacterial cultures were grown on agar slants, incubated at 37°C for 18–24 hours, and transferred into agar media to prepare seeded test plates. After the crude extract was dissolved in ethanol to a concentration of 10 µg/µL, sample discs (500 µg and 250 µg per disc), standard antibiotic discs (kanamycin (Pillar et al., 2009) and ciprofloxacin (Ghosh et al., 2008), 30 µg/disc) as a positive control, and blank discs (solvent only) were then positioned on the plates at safe distances (>15mm) to prevent overlapping zones. After incubation, the antimicrobial activity was determined by measuring the zones of inhibition (in mm).

2.6. Antioxidant Evaluation

2.6.1. DPPH Method

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a popular technique for assessing a variety of substances' antioxidant capacity. This technique relies on antioxidants' capacity to scavenge the DPPH radical (Yamauchi et al., 2024; Islam et al., 2013; Sarker et al., 2024). In order to determine the antioxidant's scavenging activity, the assay typically involves combining the test material with a DPPH solution and measuring the absorbance at 515 nm over time (Baliyan et al., 2022; Mahmud et al., 2024). A stock solution containing 500 µg/mL was prepared by precisely weighing 1 mg of *C. Indicum* extract and dissolving it in 2 mL of ethanol (99–100%). From this, ten different concentrations were prepared through serial dilution, including 250, 125 , 62.5, 31.25, 15.62, 7.81, 3.90, 1.95, and 0.97 µg/mL. Similarly, ascorbic acid solutions were prepared in the same concentration range (500 µg/mL to 0.97 µg/mL) using appropriate dilutions. For the DPPH solution, 0.00078 g of DPPH powder was dissolved in 20 mL of ethanol (99–100%) to make a 20 µg/mL DPPH solution, which was stored in a cool, dry, and dark place. In the assay of free radical scavenging activity, 2.0 mL of each sample solution (control or extractive) at varying concentrations (500.0 to 0.977 µg/mL) was mixed with 3.0 mL of the DPPH methanol solution (20 µg/mL). After a 30-minute incubation period at room temperature in the dark, the absorbance was measured at 517 nm using a UV spectrophotometer, with methanol as the blank. The deep violet color of DPPH turns yellow when combined with an antioxidant. The level of discoloration indicates the extract's scavenging ability. A lower reaction mixture absorbance indicates greater free radical scavenging capability. At an inhibiting concentration, or IC50, a sample must be able to scavenge 50% of DPPH free radicals (Baburao et al 2010).

2.7. Evaluation of Thrombolytic Activity

For the preparation of the standard, streptokinase (SK) with a potency of 1,500,000 I.U. was used. 5 mL of sterile distilled water was added to the SK to make it a concentration of 30,000 IU. From this suspension, 2.5 mL was taken and diluted with another 2.5 mL of distilled water, yielding a final concentration of 15,000 I.U. For the preparation of the sample, 100 mg and 200 mg of the ethanolic extract of *C. indicum* were each dissolved in 10 mL of distilled water to produce two concentrated solutions with final concentrations of 10 mg/mL and 20 mg/mL, respectively.

Fifteen blank centrifuge tubes were labeled (1–15) and weighed, then divided into three groups of five, designated as S10 (standard 30,000 I.U.), S20 (standard 15,000 I.U.), W (negative control), E10 (10 mg/mL extract), and E20 (20 mg/mL extract). Blood was collected from three donors and 0.5 ml of blood from each donor was added to the corresponding five tubes in each group. The tubes were incubated at 37°C for 45 minutes to allow clot formation. After clotting, the serum was carefully removed, and the initial clot weight was calculated. Next, 100 µL of each standard concentration (30,000 I.U. for S10 and 15,000 I.U. for S20), 100 µL of distilled water for W, and 100 µL of each extract concentration (E10 and E20) were added to the respective tubes. This procedure was repeated for batches 2 and 3. The tubes were incubated again at 37°C for 90 minutes to allow clot lysis. The second clot weight was then measured using the same method, and the degree of thrombolysis was calculated by measuring the difference between the initial and final clot weights, representing the extent of clot degradation. This systematic approach enabled quantitative evaluation of the clot-dissolving potential of the *C. indicum* extract and its comparison with standard thrombolytic therapy.

3. Results and Discussion

3.1. Phytochemical Screening

The initial phytochemical investigations are represented by the testing of several chemical groups found in the extract. The extract in ethanol solution was used in each assay. The tests for reducing sugar, tannins, flavonoids, saponins, gums, steroids, alkaloids, and glycosides are known as chemical group tests (Mahmud et al., 2024). **Table 1** shows that Glycosides and flavonoids were absent from the phytochemical examination, but alkaloids, steroids, gums, saponins, reducing sugars, and tannins were detected.

The phytochemical analysis of the ethanolic leaf extract of *C. indicum* showed the presence of alkaloids, steroids, gums, saponins, tannins, and reducing sugars. It has been demonstrated that these bioactive compounds have significant therapeutic advantages, including thrombolytic and antibacterial properties.

Alkaloids are well known for their antibacterial and potentially clot-dissolving properties, as shown by Mayer's and Dragendorff's experiments. Alkaloids are effective against a variety of bacteria, fungi, and other pathogens because they prevent microbial growth by interfering with the formation of cell walls, DNA replication, and enzyme function; some of these compounds have even served as models for the development of antibiotics (Amin Thawabteh et al., 2019 & Leen Othman et al., 2019). By interfering with cell membranes and enzyme function, tannins known for their ability to precipitate proteins and their antibacterial properties should enhance bacterial inhibition (Amin Thawabteh et al., 2019). The frothing test finds saponins, which support the extract's antimicrobial properties by breaking down microbial cell membranes and increasing permeability. Gums can enhance absorption and delivery even though they aren't directly active in reducing sugars. While not directly antibacterial, they might provide antioxidant support that may improve the efficiency of other chemicals (Sazia Afrin et al., 2023).

Overall, the traditional antibacterial use of *C. indicum* is supported by the presence of these phytochemicals. Further investigation into the specific functions and mechanisms of these elements is necessary, as the results indicate that the observed activity is probably the result of their combined or synergistic effects.

3.2 *In Vitro* Antimicrobial Activity

The anti-microbial activity of the ethanolic extract of the leaves of *C. indicum* was tested against a number of both gram-positive and gram-negative bacteria. To compare the results, Ciprofloxacin and Kanamycin standard discs were used. The antimicrobial activity of the extract was visualized through inhibition zone diameters presented in (**Fig. 1**).

Table 2 shows that the ethanolic extract of the leaves of *C. indicum* (250 µg/disc and 500 µg/disc) showed moderate anti-microbial activity against *Streptobacillus moniliformis*, *Salmonella enterica*, and *Streptococcus pneumoniae*.

A popular technique for screening natural materials for potential antibiotic development is the disc diffusion method, which is straightforward but efficient for determining antimicrobial activity. It measures the zone of inhibition surrounding discs using test samples on agar plates that have been infected with bacteria (Romney et al., 2018). The ethanolic extract of *C. indicum* exhibited mild antibacterial activity against both Gram-positive and Gram-negative bacteria in this study. *Streptobacillus moniliformis* showed a significant inhibitory zone of 24 mm, *Salmonella enterica* showed a significant inhibitory zone of 27 mm, and *Streptococcus pneumonia* showed a significant inhibitory zone of 19 mm at 500 µg/disc. These results show the presence of antibacterial compounds, albeit at a lower level than that of common antibiotics (Ciprofloxacin and Kanamycin).

Given that the extract included alkaloids, tannins, saponins, and steroids, all of which have antibacterial qualities, the action is in line with the phytochemical results.

3.3. Antioxidant Evaluation

3.3.1. Assay of Free Radical Scavenging Activity

2.0 mL of a methanol solution of the sample (Control/extractives) at different concentrations from 500.0 to 0.977 µg/mL were mixed with 3.0 mL of a DPPH methanol solution (20 µg/mL). After 30 minutes reaction period at room temperature in a dark place, the absorbance was measured at 517 nm against methanol as a blank by a UV spectrophotometer.

Inhibition of free radical DPPH in percent (I%) was calculated as follows-

$\{(A_o - A_1)/A_o\} \times 100$

Table 1. Different phytochemical tests and the results.

Phytoconstituents	Tests	Results
Alkaloids	Mayer's test	+
	Dragendroff's test	+
Glycosides	Bromine water test	–
	Fehling test	–
Steroids	Sulfuric acid test	+
Gums	Molisch's test	+
Flavonoids	Alkaline reagent test	–
Saponins	Frothing test	+
Reducing Sugar	Benedict's test	+
	Fehling's test	+
	Alpha-Napthol solution test	+
Tannins	$FeCl_3$ test	+

Where A_0 is the absorbance of the control reaction (containing all reagents except the test material), and A1 is the absorbance of the extract/standard.

The extract/standard concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against extract concentration. The dose-response curve for DPPH radical scavenging activity is illustrated in (**Fig. 2**). **Table 3** shows the IC50 value of ascorbic acid. **Table 4** shows the IC50 value of the Ethanolic extract of *C. indicum*. The purple-colored DPPH free radical is stabilized by turning yellow, which assesses the antioxidant's capacity to donate hydrogen atoms or electrons (Sagar et al., 2011). The IC50 value represents the concentration needed to block 50% of the DPPH radicals; lower IC50 values indicate better antioxidant potential. The decrease in absorbance at 517 nm indicates radical scavenging ability (Jolanta Flieger et al., 2020).

The ethanolic extract of *C. indicum* demonstrated high antioxidant activity, with an IC50 of 82 µg/mL compared to 14 µg/mL for ascorbic acid. The extract demonstrated a definite dose-dependent scavenging action, despite being less potent than the standard. This points to the presence of antioxidant phytochemicals that are known to aid in the neutralization of radicals, including tannins, alkaloids, saponins, and flavonoids.

These results are in line with earlier studies on antioxidants that found that Clerodendrum species had comparable capacities. The plant's traditional use in managing oxidative stress was supported by the DPPH technique's overall success in assessing antioxidant capacity.

Table 2. *In vitro* antibacterial activity of *Clerodendrum indicum* ethanolic extract.

Bacterial strains	**Diameter of zone of inhibition (mm)**			
	Kanamycin (30 µg/disc)	Ciprofloxacin (30 µg/disc)	Ethanol extract (500 µg/disc)	Ethanol extract (250 µg/disc)
Gram negative				
Streptobacillus moniliformis	24	35	24	11
Salmonella enterica	21	32	27	15
Gram positive				
Streptococcus pneumoniae	25	30	19	10

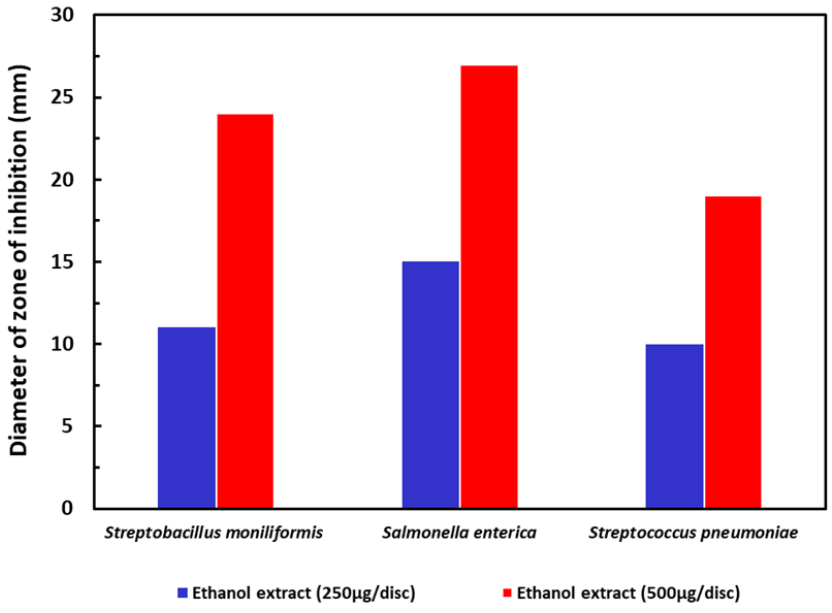


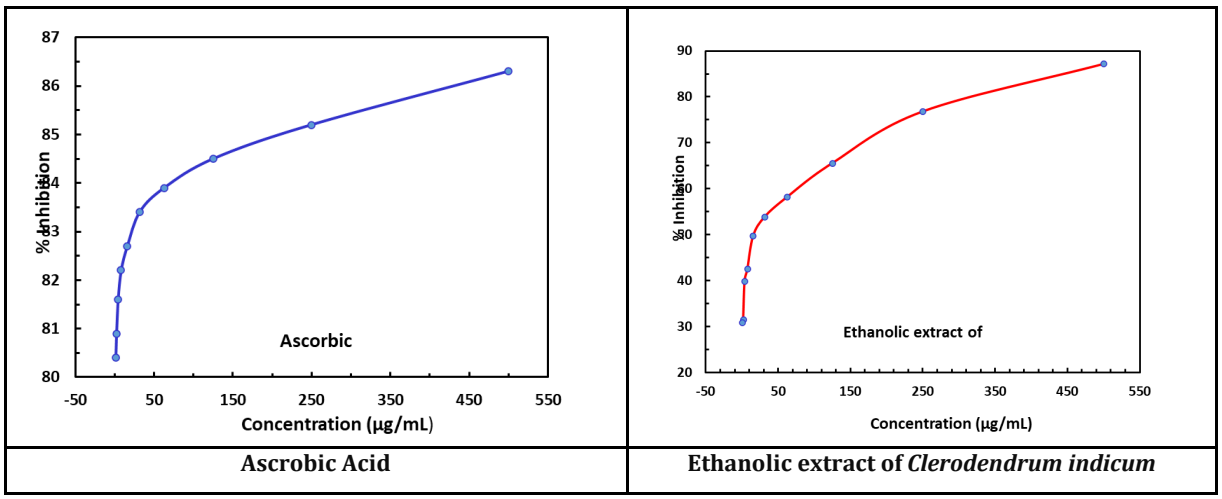
Fig. 1. The ethanolic extract of *Clerodendrum indicum* exhibited mild activity against both Gram-positive and Gram-negative bacteria, thereby lending preliminary support to its traditional use as an antimicrobial agent in coastal regions. However, this remains an initial investigation, and further comprehensive studies are required to provide more robust scientific evidence regarding its therapeutic potential.

Table 3. IC50 value of ascorbic acid.

Serial No.	Concentration (µg/mL)	Absorbance of blank	Absorbance of Ascorbic acid	% Inhibition	IC50 (µg/mL)
1	500		0.060	86.3	
2	250		0.064	85.2	
3	125		0.067	84.5	
4	62.50		0.070	83.9	
5	31.25	0.435	0.072	83.4	14
6	15.63		0.075	82.7	
7	7.81		0.077	82.2	
8	3.91		0.080	81.6	
9	1.95		0.083	80.9	
10	0.97		0.085	80.4	

Table 4. IC50 value of Ethanolic extract of *Clerodendrum indicum.*

Serial No.	Concentration µg/mL	Absorbance of blank	Absorbance of Ascorbic acid	% Inhibition	IC50 µg/mL
1	500		0.056	87.13	
2	250		0.101	76.78	
3	125		0.150	65.52	
4	62.50		0.182	58.16	
5	31.25	0.435	0.201	53.79	82
6	15.63		0.219	49.66	
7	7.81		0.250	42.53	
8	3.91		0.262	39.77	
9	1.95		0.298	31.49	
10	0.97		0.301	30.80	



Ascrobic Acid | **Ethanolic extract of *Clerodendrum indicum***

Fig. 2. Illustrates the dose-response curve for DPPH radical scavenging activity, showing a gradual decrease in absorbance with increasing concentration.

3.4. Thrombolytic Activity

Table 5 shows that the ethanoic extract of the leaves of *C. indicum* (10 mg/mL and 20 mg/mL) showed significantly higher activity than the distilled water-treated control group (W), despite being less effective than streptokinase, which represents the moderate fibrinolytic activity. A comparison of clot lysis by extract and streptokinase is shown in (Fig. 3), indicating moderate efficacy of the extract.

Table 5. *In vitro* thrombolytic activity of *Clerodendrum indicum* ethanolic extract .

Serial No.	Treatment Group	Concentration	% of Lysis of Clot
1	W(Control)		7.86
2	S_{10}	15000 IU	47.23
3	S_{20}	30000 IU	69.37
4	E_{10}	10 mg/mL	26.67
5	E_{20}	20 mg/mL	43.87

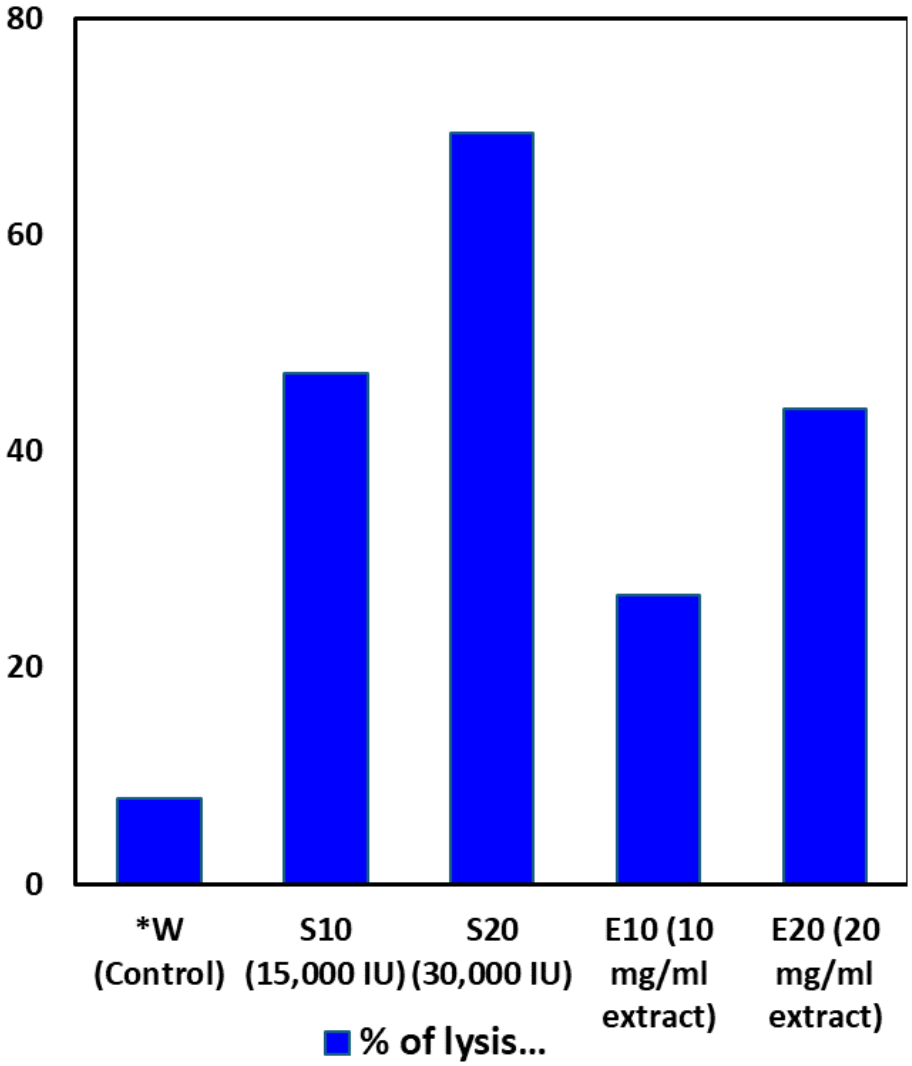


Fig. 3. The traditional thrombolytic drug streptokinase was compared to the thrombolytic activity of an ethanolic extract of *Clerodendrum indicum*. As anticipated, the control group (W) experienced only 7.86% clot lysis. Streptokinase at 30,000 I.U. (S20) exhibited the highest thrombolytic activity, resulting in 69.37% lysis of the clot, whereas the lower concentration (15,000 I.U., S10) caused 47.23% lysis. A clot lysis of 26.67% was shown by the extract at 10 mg/mL (E10) and 43.87% by the extract at 20 mg/mL (E20). This suggests that the plant's phytochemicals may have fibrinolytic activity.

C. indicum is a well-known ethnomedicinal plant traditionally used in India and Southeast Asia to treat a variety of ailments, including inflammation, blood disorders, respiratory issues, liver diseases, and skin conditions (Ashutosh Kundu et al., 2024; Harish Sharma et al., 2022). Scientific investigations have confirmed many of these uses, revealing that *C. indicum* contains a rich array of bioactive compounds such as phenolic acids, flavonoids, terpenoids, steroids, saponins, alkaloids, and glycosides, which are responsible for its notable antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective effects (Pallab Kar et al., 2019; Surya Kant Kalauni et al., 2023). Extracts from the plant have demonstrated significant antioxidant activity in various assays, and studies in animal models show that these extracts can protect against oxidative stress-induced liver injury, supporting its traditional use for liver and blood-related conditions (Pallab et al., 2019; Surya et al., 2023). The anti-inflammatory and antinociceptive properties of *C. indicum* have also been validated *in vitro*, suggesting potential for developing new anti-inflammatory drugs (M.SUSHMA et al., 2021). Additionally, the plant's phytochemicals have shown promise in promoting fibrinolysis, which aligns with its folk use for blood disorders and inflammatory conditions (M.SUSHMA et al., 2021; Ashutosh et al., 2024).

4. Conclusion

The ethanolic leaf extract of *C. indicum* demonstrated significant phytochemical diversity, including alkaloids, tannins, saponins, and steroids, which correlated with its moderate antimicrobial, antioxidant (IC50 = 82 µg/mL), and thrombolytic (43.87% clot lysis at 20 mg/mL) activities. These findings validate its traditional use and highlight its therapeutic potential. However, this study has limitations: it used only crude extracts without isolation of individual bioactive compounds, and the antimicrobial and thrombolytic evaluations were limited to *in vitro* assays. Additionally, the lack of cytotoxicity or *in vivo* studies restricts the clinical translation of these findings. Future research should focus on bioassay-guided isolation, mechanistic studies, and *in vivo* validations to elucidate specific compounds responsible for the observed effects and assess their pharmacological safety and efficacy. This will strengthen the development of *C. indicum*-derived natural therapeutics in combating oxidative stress, microbial infections, and thrombotic disorders.

Consent to Participate

We hereby confirm that this study did not involve any human participants. All experiments and procedures were conducted on animals in accordance with ethical guidelines and institutional regulations.

Conflict of Interest

No declared

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conceptualization, D.R.S. and T.R.; **methodology**, A.B.S.; **experimental observation**, D.R.S., K.G., T.R. and R.D. ; **software**, R.I., M.R.U., and I.A.Z.; **validation**, K.G., T.R. and D.R.S.; **formal analysis**, R.I., I.A.Z., and A.B.S.; **data curation**, R.I., and M.R.U.; **funding acquisition**, K.G.; **writing—original draft preparation**, S.S. and T.R., M.K.S., C.R.M., ; **writing—review and editing**, S.S. and A.B.S.; **mechanisms drawing**, S.S.; **supervision**, A.B.S.; **project administration**, A.B.S. and M.R.U.

References

Abbes, Z.; El-Abed, N.; Amri, M.; Kharrat, M.; Ahmed, S. B. H. Antioxidant and Antibacterial Activities of the Parasitic Plants Orobanche Foetida and Orobanche Crenata Collected on Faba Bean in Tunisia. J. Anim. Plant Sci. 2014, 24 (1), 310–314.

Almodaifer, S.; Alsibaie, N.; Alhoumendan, G.; Alammari, G.; Kavita, M. S. Role of Phytochemicals in Health and Nutrition. BAOJ Nutr 2017, 3, 28–34.

Álvarez-Mart, F. J.; Barrajón-Catalán, E.; Micol, V. Tackling Antibiotic Resistance with Compounds of Natural Origin: A Comprehensive Review. Biomedicines 2020, 8 (10), 405.

Amin Thawabteh et al. The Biological Activity of Natural Alkaloids against Herbivores, Cancerous Cells and Pathogens. National Library of Medicine 656, (2019).

Ashutosh Kundu et al. Development of efficient, cost-effective in vitro micropropagation technique for threatened ethnomedicinal plant Clerodendrum indicum (L.) O. Kuntze. Springer NaturE 157, (2024).

Baburao, B.; Reddy, A. R. N.; Kiran, G.; Reddy, Y. N.; Mohan. Antioxidant, Analgesic and Anti-Inflammatory Activities of Leucas Cephalotes (Roxb. Ex Roth) Spreng. Braz. J. Pharm. Sci. 2010, 46, 525–529.

Baliyan, S.; Mukherjee, R.; Priyadarshini, A.; Vibhuti, A.; Gupta, A.; Pandey, R. P.; Chang. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus Religiosa. Molecules 2022, 27 (4), 1326.

Chaudhary, P.; Janmeda, P.; Docea, A. O.; Yeskaliyeva, B.; Abdull Razis, A. F.; Modu, B.; Calina, D.; Sharifi-Rad, J. Oxidative Stress, Free Radicals and Antioxidants: Potential Crosstalk in the Pathophysiology of Human Diseases. Front. Chem. 2023, 11, 1158198.

Chen, C.; Yang, F. Q.; Zhang, Q.; Wang, F. Q.; Hu, Y. J.; Xia, Z. N. Natural Products for Antithrombosis. Evidence-Based Complement. Altern. Med. 2015, 2015 (1), 876426.

Craig, W. J. Phytochemicals: Guardians of Our Health. J. Am. Diet. Assoc. 1997, 97 (10), S199–S204.

De, S.; Dey, Y. N.; Ghosh, A. K. Phytochemical Investigation and Chromatographic Evaluation of the Different Extracts of Tuber of Amorphaphallus Paeoniifolius (Araceae). Int J Pharm Biol Res 2010, 1 (5), 150–157.

Devor, A. W. Carbohydrate Tests Using Sulfonated α-Naphthol. J. Am. Chem. Soc. 1950, 72 (5), 2008–2012.

Diwan, D.; Usmani, Z.; Sharma, M.; Nelson, J. W.; Thakur, V. K.; Christie, G.; Molina, G.; Gupta. Thrombolytic Enzymes of Microbial Origin: A Review. Int. J. Mol. Sci. 2021, 22 (19), 10468.

Emran, T. B.; Rahman, M. A.; Uddin, M. M. N.; Rahman, M. M.; Uddin, M. Z.; Dash, R.; Layzu, C. Effects of Organic Extracts and Their Different Fractions of Five Bangladeshi Plants on in Vitro Thrombolysis. BMC Complement. Altern. Med. 2015, 15, 1–8.

Evans Pharmacognosy., 15th ed.; 2002.

Ghosh, A.; Das, B. K.; Roy, A.; Mandal, B.; Chandra, G. Antibacterial Activity of Some Medicinal Plant Extracts. J. Nat. Med. 2008, 62, 259–262.

Gilgun-Sherki, Y.; Melamed, E.; Offen, D. Oxidative Stress Induced-Neurodegenerative Diseases: The Need for Antioxidants That Penetrate the Blood Brain Barrier. Neuropharmacology 2001, 40 (8), 959–975.

Harborne, A. J. Phytochemical Methods a Guide to Modern Techniques of Plant Analysis. Springer Sci. Bus. Media 1998.

Harish Sharma, Ajay Kumar Verma & Krishna Priya Jha. A Brief Study on Medicinal Plant Clerodendrum Species: A Review Article. International Journal of Pharmaceutical Research and Applications 7, 440–442 (2022).

Houldsworth, A. Role of Oxidative Stress in Neurodegenerative Disorders: A Review of Reactive Oxygen Species and Prevention by Antioxidants. Brain Commun. 2024, 6 (1), 356.

Hussain, T.; Tan, B.; Yin, Y.; Blachier, F.; Tossou, M. C.; Rahu, N. Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? Oxid. Med. Cell. Longev. 2016, 2016 (1), 7432797.

Islam, M. A.; Alam, F.; Ibrahim Khalil, M.; Haryo Sasongko, T.; Hua Gan, S. Natural Products towards the Discovery of Potential Future Antithrombotic Drugs. Curr. Pharm. Des. 2016, 22 (20), 2926–2946.

Islam, M. K.; Mahmud, I.; Saha, S.; Sarker, A. B.; Mondal, H.; Monjur-Al-Hossain, A. S. M.; Anisuzzman, M. Preliminary Pharmacological Evaluation of Alocasia Indica Schott Tuber. J. Integr. Med. 2013, 11 (5), 343–351.

Jolanta Flieger & Michał Flieger. The [DPPH●/DPPH-H]-HPLC-DAD Method on Tracking the Antioxidant Activity of Pure Antioxidants and Goutweed (Aegopodium podagraria L.) Hydroalcoholic Extracts. MDPI (2020).

Kar, P.; Goyal, A. K.; Das, A. P.; Sen, A. Antioxidant and Pharmaceutical Potential of Clerodendrum L.: An Overview. Int. J. Green Pharm. IJGP 2014, 8 (4).

Kubatka, P.; Mazurakova, A.; Koklesova, L.; Samec, A.; Sokol, J.; Samuel, S. M.; Kudela, E.; Biringer, K.; Bugos, O.; Pec, M.; Link, B. Antithrombotic and Antiplatelet Effects of Plant-Derived Compounds: A Great Utility Potential for Primary, Secondary, and Tertiary Care in the Framework of 3P Medicine. EPMA J. 2022, 12 (3), 407–431.

Kunwar, A.; Priyadarsini, K. I. Free Radicals, Oxidative Stress and Importance of Antioxidants in Human Health. J Med Allied Sci 2011, 1 (2), 53–60.

Lamponi, S. Bioactive Natural Compounds with Antiplatelet and Anticoagulant Activity and Their Potential Role in the Treatment of Thrombotic Disorders. Life 2021, 11 (10), 1095.

Leen Othman, Ahmad Sleiman & Roula M. Abdel-Massih. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. Frontiers (Boulder) 10, (2019).

Lei, W.; Li, X.; Li, S.; Zhou, F.; Guo, Y.; Zhang, M.; Jin, X.; Zhang, H. Targeting Neutrophils Extracellular Traps, a Promising Anti-Thrombotic Therapy for Natural Products from Traditional Chinese Herbal Medicine. Biomed. Pharmacother. 2024, 179, 117310.

Lichota, A.; Szewczyk, E. M.; Gwozdzinski, K. Factors Affecting the Formation and Treatment of Thrombosis by Natural and Synthetic Compounds. Int. J. Mol. Sci. 2020, 21 (21), 7975.

Lokesh, S. T.; Basaiah, T.; Akarsh, S. Qualitative and Quantitative Studies of Reducing Sugars, Protein Contents and Antifungal Activity of Secondary Metabolites of Claviceps Purpurea. Plant Arch. 2020, 20 (2), 5139–5145.

M.SUSHMA, S.LAHARI, A. MOUNIKA & K.E.SAILAJA. PHYTOCHEMICAL SCREENING & IN-VITRO EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF CLERODENDRUM INDICUM ROOTS. World Journal of Current Medical and Pharmaceutical Research 3, (2021).

Madhuranga, H. D. T.; Praba Jalini Wijekumar, D. N. A.; Samarakoon, W. Herbal Remedies That Can Be Used to Treat Type 1 Hypersensitivity Reactions Associated with Allergic Rhinitis and Asthma in Sri Lanka-A Systematic Review. JAHM 2023, 9 (1), 29–39.

Mahmud, I.; Rahman, M. S.; Sarker, A. B.; Parvez, M. R. Assessment of Phytochemical, Antioxidant, and Antibacterial Properties of Xylocarpus Granatum Leaves. J. Med. Plants Stud. 2024, 12 (1), 191–196.

Mahmud, I.; Rahman, M. S.; Sarker, A. B.; Parvez, M. R. Assessment of Phytochemical, Antioxidant, and Antibacterial Properties of Xylocarpus Granatum Leaves. J. Med. Plants Stud. 2024, 12 (1), 191–196.

Mazumder, T.; Salam, M. A.; Mitra, S.; Hossain, S.; Hussain, M. S. Current Antithrombotic Therapies and Prospects of Natural Compounds in the Management of the Thrombotic Disorder. Visag. Publ. House 2022, 3 (2), 134–175.

Omara, T.; Kiprop, A. K.; Kosgei. Intraspecific Variation of Phytochemicals, Antioxidant, and Antibacterial Activities of Different Solvent Extracts of Albizia Coriaria Leaves from Some Agroecological Zones of Uganda. Evidence-Based Complement. Altern. Med. 2021, 2021 (1), 2335454.

Pallab Kar et al. The antioxidant rich active principles of Clerodendrum sp. controls haloalkane xenobiotic induced hepatic damage in murine model. Saudi J Biol Sci 26, 1539 –1547 (2019).

Parekh, J.; Chanda, S. In Vitro Antimicrobial Activity of Trapa Natans L. Fruit Rind Extracted in Different Solvents. Afr. J. Biotechnol. 2007, 6 (6).

Pillar, C. M.; Goby, L.; Draghi, D.; Grover, P.; Thornsberry, C. Evaluating the in Vitro Susceptibility of Bovine Mastitis Pathogens to a Combination of Kanamycin and Cefalexin: Recommendations for a Disk Diffusion Test. J. Dairy Sci. 2009, 92 (12), 6217–6227.

Prakash, D.; Gupta, C.; Sharma, G. Importance of Phytochemicals in Nutraceuticals. J. Chin. Med. Res. Dev. 2012, 1 (3), 70–78.

Priyank, I.; Shonu, J.; Gaurav, J.; Dubey, B. K. Pharmacognostic Evaluation and Phytochemical Screening of Leucas Cephalotes. Int. J. Phytopharm. 2011, 1, 15–26.

Rahman, S. M.; Mony, T.; Ahammed, K.; Naher, S.; Haque, M. R.; Jui. Qualitative Phytochemical Screening and Evaluation of Analgesic and Antidiarrheal Activity of Ethanolic Extract of Leucas Cephalotes Leaves. J Pharmacogn Phytochem 2018, 7, 1484–1492.

Romney M Humphries et al. The Continued Value of Disk Diffusion for Assessing Antimicrobial Susceptibility in Clinical Laboratories: Report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. Journal of clinical microbiology (2018).

Sagar B Kedare & R P Singh. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 48, 412–422 (2011).

Sarker, A. B.; Rahman, M. A.; Uddin, A.; Sarkar, M. K.; Sheikh, S.; Islam, M. T. DPPH Radical Scavenging and Antibacterial Potentials of Leucas Cephalotes Ethanolic Extract. World J. Pharm. Pharm. Sci. 2024, 13 (10), 1439–1451.

Sazia Afrin, Kamrul Hasan, Mahmudunnabi Mithu & Mohibul Hasan. Evaluation of Phytochemical Screening, Antioxidant and Anti-Diarrheal Activities of Traditional Medicinal Shrub in Albino Mice. Global Veterinaria 32–38 (2023).

Sharma, G.; Srivastava, A. K.; Prakash, D. Phytochemicals of Nutraceutical Importance: Their Role in Health and Diseases. Pharmacologyonline 2011, 2, 408–427.

Shubham, S.; Mishra, R.; Gautam, N.; Nepal, M.; Kashyap, N.; Dutta. Phytochemical Analysis of Papaya Leaf Extract: Screening Test. EC Dent. Sci. 2019, 18 (3), 485–490.

Sidde, L. S. L.; Malathi, S.; Malathi, S. S. A Brief Review on Clerodendrum Indicum. Int. J. Indig. Herbs Drugs 2018, 3 (5), 1–4.

Siddik, A. S. N. U.; Alam, S.; Borgohain, R.; Chutia, P. Antioxidant

Properties of Clerodendrum Species Found in North East India: A Review. J Pharmacogn Phytochem 2021, 10 (4), 390-394.

Sies, H. E. Physiological Society Symposium: Impaired Endothelial and Smooth Muscle Cell Function in Oxidative Stress. Exp Physiol 1997, 82 (2), 291–295.

Sofowora, A. Screening Plants for Bioactive Agents. Med. Plants Tradit. Med. Afr. 2nd Ed Spectr. Books Ltd Sunshine House Ib. Niger. 1993, 134–156.

Surya Kant Kalauni, Sushil Kumar Mahato & Lekha Nath Khanal. Phytochemical Studies and Toxicity Evaluation of Selected Medicinal Plants from Sarlahi District, Nepal. Journal of Plant Resources (2023).

Sushma, M.; Lahari, S.; Mounika, A.; Sailaja, K. E. Phytochemical Screening & In-Vitro Evaluation of Anti-Inflammatory Activity of Clerodendrum Indicum Roots. World J Curr Med Pharm Res 2021, 3 (6), 140–143.

Tan, B. L.; Norhaizan, M. E.; Liew, W. P. P.; Sulaiman Rahman, H. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front. Pharmacol. 2018, 9, 1162.

Vazhappilly, C. G.; Ansari, S. A.; Al-Jaleeli, R.; Al-Azawi, A. M.; Ramadan, W. S.; Menon, V.; Hodeify, R.; Siddiqui, S. S.; Merheb, M.; Matar, R.; Radhakrishnan, R. Role of Flavonoids in Thrombotic, Cardiovascular, and Inflammatory Diseases. Inflammopharmacology 2019, 27, 863–869.

Yamauchi, M.; Kitamura, Y.; Nagano, H.; Kawatsu, J.; Gotoh, H. DPPH Measurements and Structure—Activity Relationship Studies on the Antioxidant Capacity of Phenols. Antioxidants 2024, 13 (3), 309.