



Exploring The Antioxidant, Thrombolytic, Membrane Stabilizing, Antimicrobial, Antidiarrheal And Analgesic Activity Of Clerodendrum Wallichii Leaves

Md Rawfur Rahim¹, Sonia Zaman², Israt Jahan bulbul³, Mozahidul Haque Sohan⁴, Effat Ara⁵, Jhorora
Tabassum Shanta⁶

¹Student, Southeast University, Dhaka, Bangladesh.

²Assistant Professor, Southeast University, Dhaka, Bangladesh.

³Assistant Professor, Southeast University, Dhaka, Bangladesh.

⁴Student, Southeast University, Dhaka, Bangladesh.

⁵Student, Southeast University, Dhaka, Bangladesh.

⁶Student, Southeast University, Dhaka, Bangladesh.

Abstract: This study delves into the longstanding relationship between humans and plants, where the utilization of plants as remedies for human ailments dates back to the dawn of civilization. This research aims to bridge the gap between traditional wisdom and modern science by obtaining experimental data on the medicinal plant *C. wallichii*. The study's objectives encompass the identification of phytochemical and antioxidant activities, membrane stabilizing properties, antimicrobial potential, thrombolytic activity, and antioxidant capacity of *C. wallichii*. This study conducted a comprehensive analysis of the methanol extract of *C. wallichii* leaves, aiming to uncover its phytochemical composition and therapeutic potential. Notably, the extract exhibited significant antioxidant capacity, with a flavonoid content of 66.67 mg of quercetin equivalents per gram of dried extract, and demonstrated reducing power comparable to ascorbic acid. However, antimicrobial assays showed limited inhibitory effects against various pathogens, urging further investigation to enhance its antimicrobial properties. The findings underscore the need for in-depth chemical and pharmacological investigations to isolate bioactive compounds and assess safety and efficacy, offering promising avenues for the development of immunomodulatory therapies, antioxidant supplements, antimicrobial agents, thrombolytic treatments, and membrane protective strategies.

Keywords: *C. wallichii*, Thrombolytic, Vitro Antioxidant, Flavonoid, Actephila, Antimicrobial

1. Introduction

Plants and human are inseparable. The utilization of plants to treat human diseases is as aged as the expansion of human civilization itself. From the starting of human civilization, medicinal plants have played an important role for the welfare of human beings. In the development of human health, the use of natural product is tremendous from the ancient time. In the early stages, people chewed plant herbs to mitigate pain and used wrapped leaves for wound healing. Ancient people in search for nourishment and to cope effectively with human sufferings started to differentiate those plants appropriate for medicinal use with particular pharmacological activity from others (Shakya, 2016). In recent years, tremendous changes have observed in the preliminary health care sector through the improvement of medical science and technology, but still 400 cores of men are totally reliant on herbal medicine. It is reported that even in the prosperous countries 25% of the prescription drugs come from natural plant sources and 75-80% of people in the world use herbal drugs for prime health facilities because of their better compatibility with human body (Kifayatullah et al., 2015). Plant and men are inseparable. From the early stage of human civilization plant play an important role for the welfare of human beings. When a plant is designated as “Medicinal” it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medical preparation. Medicinal plant may therefore be defined as a group of plants that possess some special properties of virtues that qualify them as articles of drugs and therapeutic agents and are used for medicinal purpose. Plants have traditionally been used for treatment of human and livestock ailments in Ethiopia by different ethnic and social groups. Knowledge can arise from scientific or traditional sources. Traditional knowledge has been described as a cumulative body of knowledge, practice and belief, evolving through adaptive processes and handed over through generations by cultural transmission. Traditional medicine is used throughout the world as it is heavily dependent on locally available plant species and plant-based products and capitalizes on traditional wisdom repository of knowledge. The wide spread use of traditional medicine could be attributed to cultural acceptability, economic affordability and efficacy against certain type of diseases as compared to modern medicines. Thus, different local communities in countries across the world have indigenous experience in various medicinal plants where they use their perceptions and experience to categorize plants and plant parts to be used when dealing with different ailments. Bangladesh Possess a rich flora of medicinal plant. A great variety of medicinal plants grow in its forest, jungles waste land and in the road sides. Out of the estimated 5700 species of higher plant growing in this country. Most of the people of our country are poor. They can't buy medicine because of their economic problem. So, they are totally depending on plant for their treatment especially in remote rural areas, and herbal medicine is very much cheap and easily available. Furthermore, many more medicinal plants are used traditionally of various disease and ailments but very few of them are supported by scientific evidence. Above those reasons we select medicinal plant for our study because our study to obtain experimental data for the selected plant against particular disease. These data will support the traditional use of medicinal plant to treat various disease.

2. Aims of the Study

The aim is overarching target which needs to be fulfilled by the end of the research. Also, make sure there is only one research aim in a single research study.

Plant medicines are used as both internal and external treatments of human disease. Many plants have anti-bacterial, anti-viral, anti-inflammatory, anti-cancer, immune stimulatory and antioxidant properties as well as compounds that effect specific organs and systems in direct ways. Since ancient time plants have served as a natural source of treatments and therapies such as aspirin, quinine and coffee. Plants improved through the use of biotechnology can produce the essential proteins for innovative treatments for diseases such as cancer, HIV, heart disease, diabetes, Alzheimer's disease, kidney disease, Crohn's disease, cystic fibrosis multiple sclerosis, spinal cord injuries, Hepatitis C, chronic obstructive pulmonary disorder (COPD), obesity, arthritis and iron deficiency. Because of such great opportunities in medicinal plants, continuous approaches are going on to isolate various therapeutically active compounds. As part of these processes I liked to work on a plant called *C.wallichii* current study focuses on the analysis of biological activity of crude methanolic extract of these plant.

3. Objectives of the Study

1. To identify phytochemical activity of leaf extracts of *C.wallichii*
2. To identify antioxidant activity of leaf extracts of *C.wallichii*
3. To identify membrane stabilizing activity of leaf extracts of *C.wallichii*
4. To find out antimicrobial activity of leaf extracts of *Boehmeria macrophylla*
5. To identify thrombolytic activity of leaf extracts of *C.wallichii*
6. To identify the antioxidant activity of *C.wallichii*

4. Literature Review

A short number of studies were done on this plant previously. The reported studies are shown below:

Shrivastava & Patel (2007) studied and reported that the major chemical constituents of the genus *Clerodendrum* are phenolics, steroids, di- and triterpens, flavonoids, volatile oils etc.

The whole plant extract of *C. wallichii* Merr. has been found to be effective in reducing pain in mice. In addition, the crude extract of *C. wallichii* and its different fractions showed prominent antioxidant and cytotoxic activities. Further studies are needed to isolate the bioactive compounds from this plant extracts which are responsible for the bioactivities (Ahmed et al., 2019).

Hydro-alcohol extract showed significant ($p < 0.05$) anti-diarrheal activity. Furthermore, amongst various fractions of hydro-alcohol extract, only chloroform fraction exhibited potent anti-diarrheal activity ($p < 0.05$). The findings of current suggest that *C. wallichii* possesses anti-diarrheal activity and could serve as a potential source for the treatment of diarrhea (Bainsal et al., 2022).

Shrivastava & Patel (2007) reported that the genus *Clerodendrum* is being used as medicines system for the treatment of various life threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension.

5. Research Methodology

5.1 Collection and Identification of plant materials

The fresh leaves of *C. wallichii* were collected from Lawachara National Park at Moulvibazar in Bangladesh in April, 2021 and identified by an expert taxonomist of Bangladesh National Herbarium, Dhaka. A voucher specimen with accession No. DACB-46005 has been deposited in Bangladesh National Herbarium, Dhaka, Bangladesh.



Figure 1: Collected Plant Leaves

5.2 Drying and Grinding

The collected plants Leaves was separated from undesirable materials or plants or plant parts. Then the leaves were washed properly to remove dirty materials and shade dried for several days with occasional sun drying. The dried plants were ground into coarse powder by a blender. The powder was stored in an airtight container and kept in a cool, dark and dry place until the next procedure.



Figure 2: Dried Plant Leaves

5.3 Extraction

Extraction as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. Crude plant drugs find their way in modern medicine system through continuous extraction followed by different isolation techniques and different pharmacological tests. Chemical constituents from crude plant can be extracted by following two extraction procedures: (i) Cold extraction and (ii) Hot extraction. In our current study we used cold extraction method.

5.4 Cold extraction procedure

In this process powdered plant materials are submerged in a suitable solvent or solvent system in an air tight flat bottom container for seven days, with occasional shaking and stirring. The major portion of plant materials will be dissolved in the solvent. Then the solvent was separated from dispersed materials and evaporated to get desired extract.

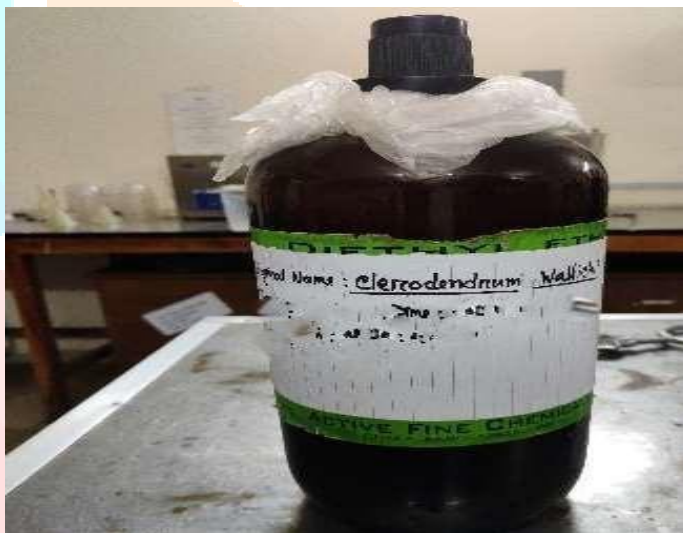


Figure 3: Plant materials soaked

5.5 Cold extraction of the Plant materials

Powdered plant materials having a weight of about 150 gm were taken in a reagent bottle and soaked in about 800 ml methanol. The bottle with its contents was sealed and kept for a period of about 10 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper. The filtrates were concentrated with a rotary evaporator under reduced pressure at 50°C temperature to afford crude extract.



Figure 4: Rotary evaporator & Filter

Then kept on water bath, being semisolid. It rendered a gummy or semisolid concentrates and were designated as crude extracts.



Figure 5: Final crude Extract

5.6 Schematic Diagram of Cold Extraction

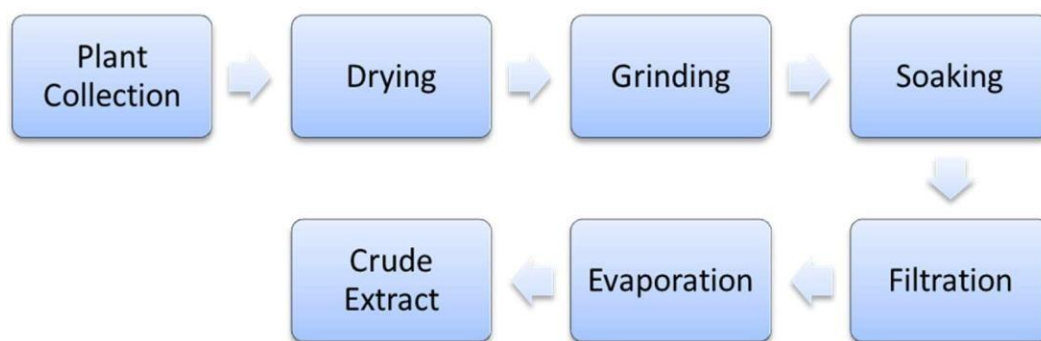


Figure 6: Schematic Diagram of Cold Extraction

5.7 Anti-Microbial Activity Test (Disk Diffusion Method)

Worldwide, infectious disease is one of main causes of death accounting for approximately one half of all deaths in tropical countries. The increases are attributed to increases in respiratory tract infections and HIV/AIDS. This test measures the ability of each test sample to inhibit the in vitro fungal and bacterial growth. This ability may be estimated by any of the following three methods.

(a) Disc diffusion method, (b) Serial dilution method and (c) Bioautographic method

5.8 Determination of Anti-diarrheal Activity (in-vivo)

Castor Oil Induced Diarrhoea

The method described by Awouters et al. was followed for this study.

1. Swiss albino mice of either sex were fasted for 18 h with free access to water and

2. Grouping: Each group contains 4 mice

- a. Group I: Normal control group: given saline water
- b. Group II: Standard control group: is treated with loperamide (10mg/kg)
- c. Group III: Drug control group: is treated with extract (100mg/kg)
- d. Group IV: Drug control group: is treated with extract (200mg/kg)

3. Normal control group: given saline water and then 0.5 ml castor oil orally. Each animal of the control group was then housed in a separate transparent cage in which the floor is lined with white paper. The paper was changed every hour for a total of four hours. After 30 minutes of castor oil administration i) the onset of diarrhoea, ii) number of wet stools, iii) total number and the total weight of faecal output were recorded.

4. Standard control group: is treated with loperamide (10mg/kg). One hour after treatment with standard loperamide, diarrhoea was induced by oral administration of 0.5 ml castor oil to each mouse. After 30 minutes of castor oil administration i) the onset of diarrhoea, ii) number of wet stools, iii) total number and the total weight of faecal output were recorded.

5. Drug control group: is treated with extract (100mg/kg and 200mg/kg). One hour after treatment with extract (100mg/kg and 200mg/kg), diarrhoea was induced by oral administration of 0.5 ml castor oil to each mouse. After 30 minutes of castor oil administration i) the onset of diarrhoea,

ii) number of wet stools, iii) total number and the total weight of faecal output were recorded.

Table 1: Drug Control Group

Group	Treatment. dose,route	Total number of feces	Number of wet stools	Total weight of faecal output
i	.			
ii	Loperamide, 10mg/kg, p.o.			
iii	Extract, 100 mg/kgb.w.			
iv	Extract, 100 mg/kg			

$$\% \text{ of inhibition} = \frac{M_o - M}{M_o} \times 100$$

Where,

MO = mean defection of control

M = mean defection of extract / standard

5.9 Analgesic activity

5.9.1 Acetic acid induced writhing Method

Principle

In this method (Ahmed et al., 2001) acetic acid (0.7% v/v) is administered intra-peritoneal to the experimental animals to create pain sensation. As a result, the animals squirms their body at regular interval out of pain. This squirm or contraction of the body is termed as “writhing”. As long as the animals feel pain, they continue to give writhing. Each writhing is counted and taken as an indication of pain sensation. Any substance that has got analgesic activity is supposed to lessen the number of writhing of animals within in a given time frame and with respect to the control group. The writhing inhibition of positive control was taken as standard and compared with test samples and control. As positive control, any standard NSAID drug can be used. In the present study, Indomethacin was used to serve the purpose.

Methods & Materials

Group I: Normal control

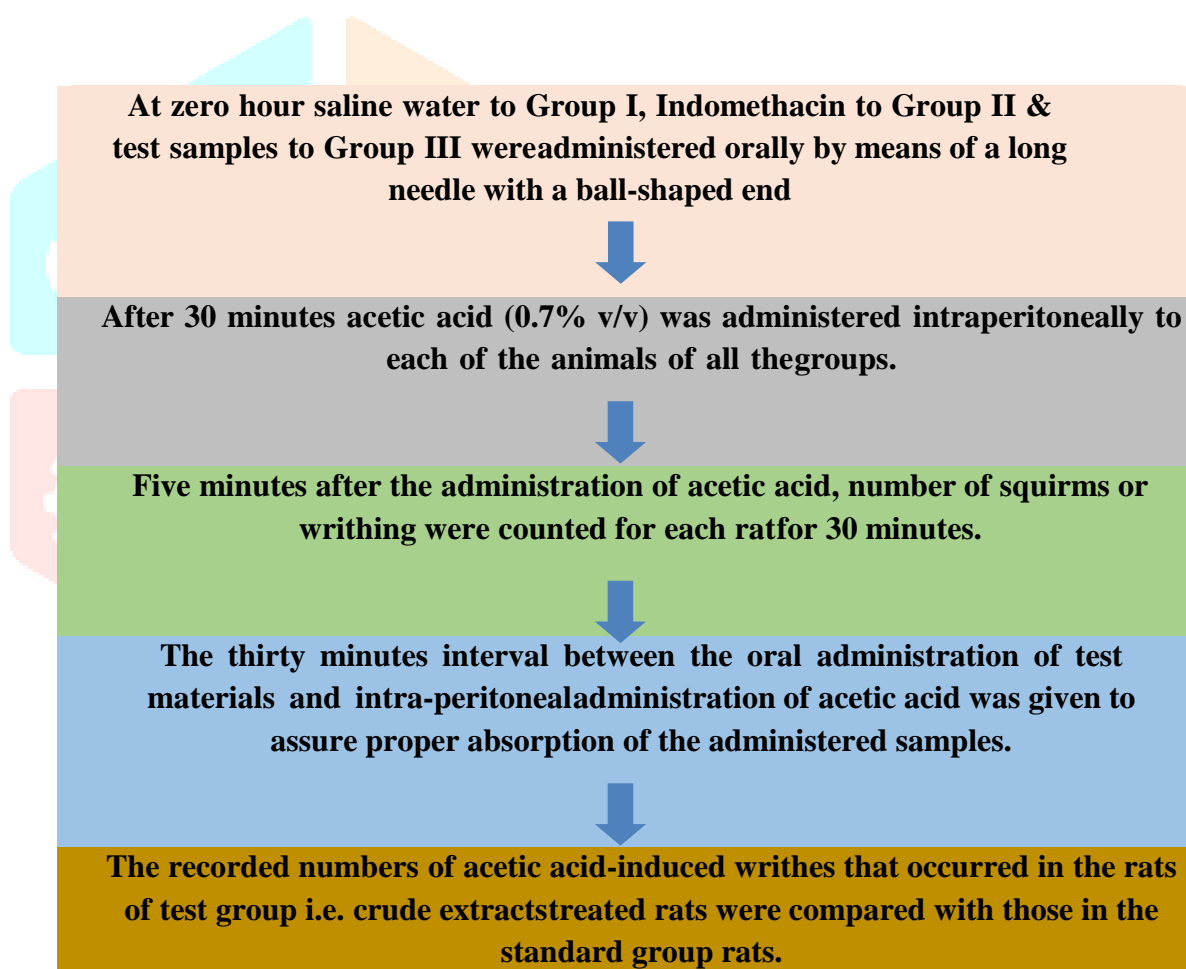
- Normal control is treated with 1% tween 80 in saline water (10 ml/kg p.o)
- After 30 minutes 0.7% Acetic acid is injected intra-peritoneally at a dose of 0.1ml/ 10g body weight.
- 0.7% acetic acid is prepared from 100% acetic acid solution.
- Then writhing response is observed for 30 minutes.

Group II: Positive control

- As a positive control indomethacine (10mg/kg) is administered orally
- Indomethacine is prepared in saline water by using tween 80.
- After 30 minutes 0.7% Acetic acid is injected intraperitoneally at a dose 0.1ml/ 10g body weight.
- Then writhing response is observed for 30 minutes.

Group III: Drug control

- The plant extract is given in two different doses 100mg/kg and 200mg/kg body weight orally
- Extract solution is prepared by dissolving the extract in saline water with tween 80.
- After 30 minutes 0.7% Acetic acid is injected intraperitoneally at a dose 0.1ml/ 10g body weight.
- Then writhing response is observed for 30 minutes.

**Figure 7: Experimental Flowchart**

$$\% \text{ of inhibition} = \frac{N - N_t}{N} \times 100$$

N= Average No. of writhing of normal control group.

N^t = Average no. of writhing of Drug control group/ Std. control group.

Table 2: Effect of the extracts on Acetic acid induced writhing method in animals.

Groups	Treatment	Dose, route	No. of writhing	% of inhibition of writhing
Group-I (Control)	1% tween 80 in saline water	1 ml, p.o		
Group-II (Std)	Indomethacin	10 mg/kg, p.o		
Group-III (Extract)		100 mg/kg, p.o		
Group-III (Extract)		200mg/kg, p.o		

5.9.2 Analgesic Activity: Formalin induced Licking and Biting Method

Group I: Normal control

- Normal control is treated with 1% tween 80 in saline water (10 ml/kg p.o)
- After 60 minutes 20µl of 5% formalin is injected into the dorsal surface of right hind paw.
- 5% formalin is prepared from 100% formalin solution.
- Then the licking of the injured paw will be counted for 30 minutes into two phases. Early Phase (0-5 minutes) and Late Phase (15-30 minutes).

Group II: Positive control

- The plant extract is given in two different doses 100mg/kg and 200mg/kg body weight orally
- Extract solution is prepared by dissolving the extract in saline water with tween 80.
- After 60 minutes 20µl of 5% formalin is injected into the dorsal surface of right hind paw.
- Then the licking of the injured paw will be counted for 30 minutes into two phases.

Table 3: Effect of the extracts on hindpaw licking in the formalin test in animals.

Groups	Treatment	Dose, route	No. of licking in Early phase	No. of Licking in Late phase	% protection of early phase	% Protection of late phase
Gr-I (Control)	1% tween 80 in saline water	1 ml, p.o				
Gr-II (Std)	Indomethacin	10 mg/kg, p.o				
Gr-III (Ext)	MEHS	100 mg/kg, p.o				
Gr-IV (Ext)	MEHS	200 mg/kg, p.o				

$$\% \text{ of inhibition} = \frac{N - N_t}{N} \times 100$$

N= Average No. of licking of normal control group.

N t= Average no. of licking of Drug control group/standard group

6. Results & Discussion

6.1 Determination of Membrane Stabilizing Activity

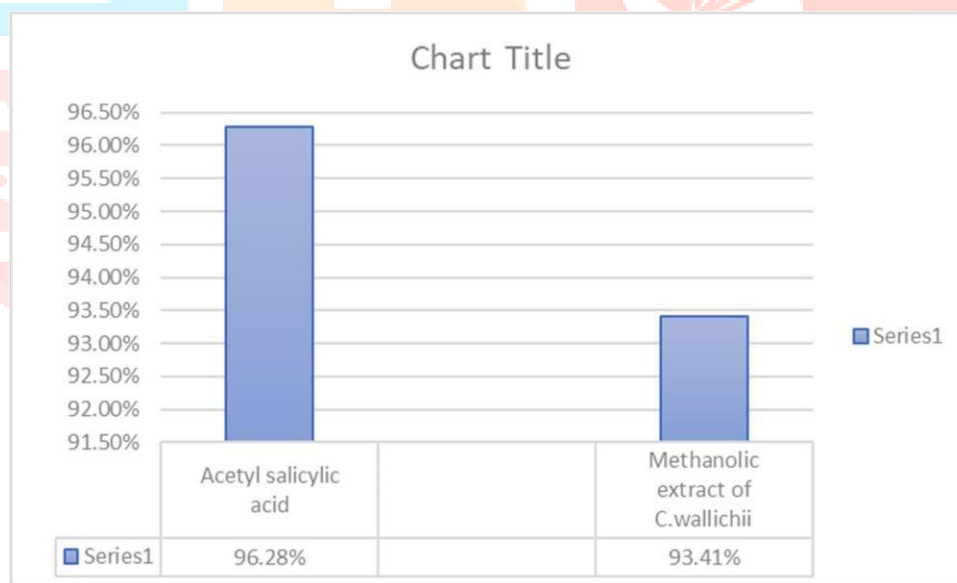


Figure 8: Column Representation of Membrane Stabilizing Activity

Table 4: Data collected for Membrane Stabilizing

Group	Dose	Absorbance	Percent of inhibition of haemolysis
Control	-	1.548	-
Acetyl salicylic acid	0.1m g/ml	0.057	96.28%
MECW	1.0m g/ml	0.102	93.41%

6.2 Determination of Thrombolytic Activity

As a part of discovery of cardio protective drugs from natural sources, the extractive of *I Wahlenbergia hirsuta*(wp) either assess or not for trombolytic activity and the result are presented on table 11. Addition of 100 μ l, a positive control (30,00 I.U) (S-kinase popular pharma) to the clots and subsequent incubation for 90 minutes at 37°C showed lysis of clot. On the other hand, plant extract was treated as negative control which exhibited a negligible percentage of lysis of clot 9.260. The mean difference in clot lysis percentages between positive and negative control was found very significant.

Table 5: Data collected for thrombolytic activity

Amount of Leaf extract	Weight of empty eppendorf tube	Weight of clot containing eppendorf tube before clot disruption	Weight of clot containing eppendorf tube after clot disruption	Weight of clot before lysis	Weight of lysis clot	% of lysis
	W _{1g}	W _{2g}	W _{3g}	W ₄ = W ₂ -W ₁ g	W ₅ =W ₂ -W ₃ g	(W ₅ /W ₄)* 100
4mg	1.112	2.018	1.687	0.906	0.34	37.53%
Blank	1.113	2.00	0.890	1.750	0.890	36.57%
SK	1.110	1.92	0.810	1.882	0.810	41.02%

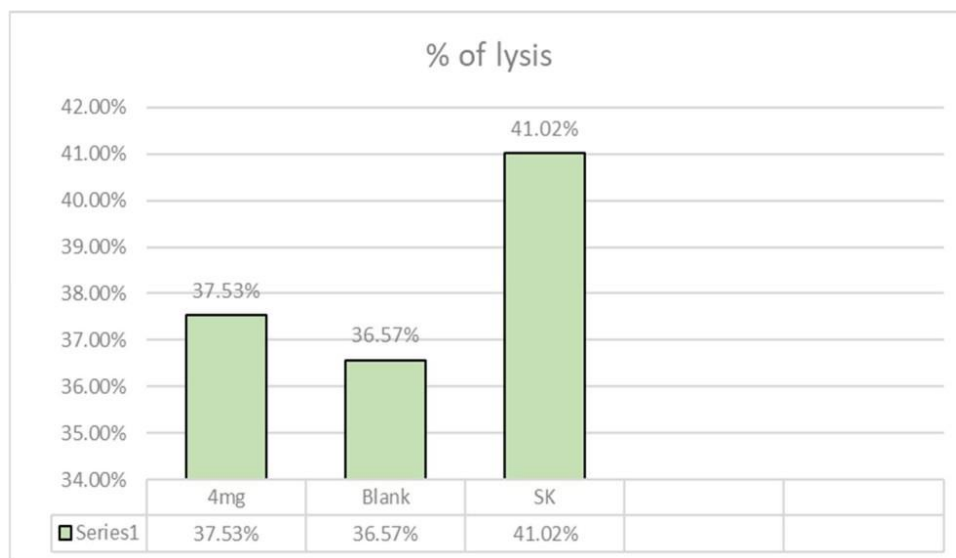


Figure 9: Column Representation of Thrombolytic Activity

6.3 Results of In Vitro Antioxidant activity test

6.3.1 Ferric reducing power capacity

The reducing power of the plant extracts were measured based on the Fe^{3+} /ferricyanide to ferrous reduction at 700 nm at different concentrations (Oyaizu, 1986). The Fe^{3+} to Fe^{2+} reducing capacity of the plant extracts, like the antioxidant activity of the extracts, showed a dose dependent increase in reducing power. Reducing power is correlated with the presence of reductones in a sample (Duh, 1998). Reductones donate a hydrogen atom to break the free radical (Gordon, 1990) and gives its antioxidant effect. The reductive capabilities of methanolic extract of leaves of *C. wallichii* and standard ascorbic acid are shown in table:

Table 6: The reductive capabilities of methanolic extract of leaves of *C. wallichii* and standard ascorbic acid

Concentration ($\mu\text{g/ml}$)	Absorbance of Standard
25	0.315
50	0.504
100	0.818
200	1.316
250	1.538
500	2.498

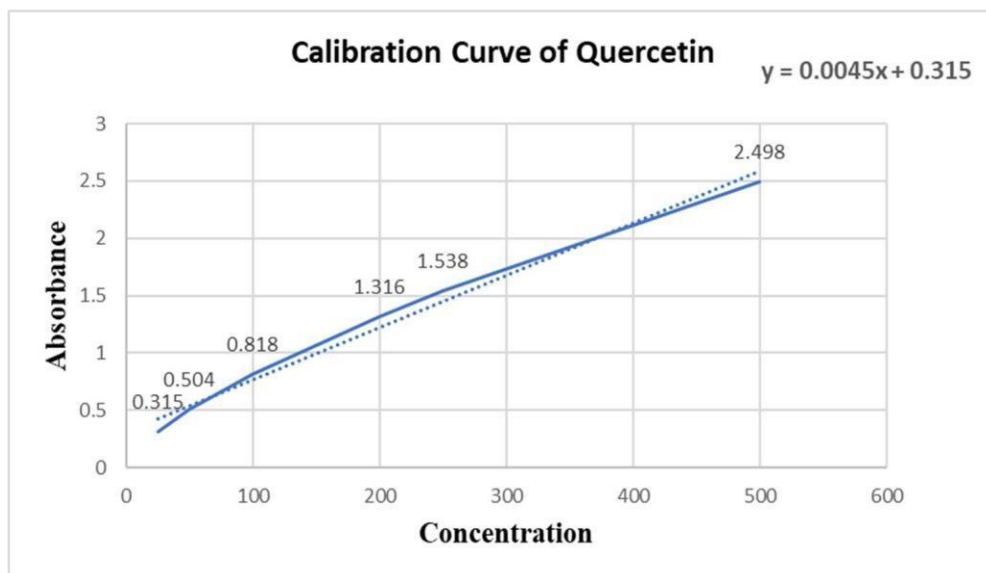


Figure 10: Standard curve of ascorbic acid for the determination of reducing power capacity of crude extract

Table 7: Reducing power capacity of standard Ascorbic acid and methanol extract of *C.wallichii* at different concentrations.

Concentration	Abs. of sample	m=Wt. of Plant Extract	c	c	V	A=(c*V)/m
µg/ml		gm	µg/ml	mg/ml	ml	mg/gm
200	0.301	0.00020	3.111	3.11×10^{-03}	2.0	31.11
250	0.349	0.00025	7.555	7.55×10^{-03}	2.0	60.44
500	0.607	0.0005	64.888	0.06488	2.0	259.52

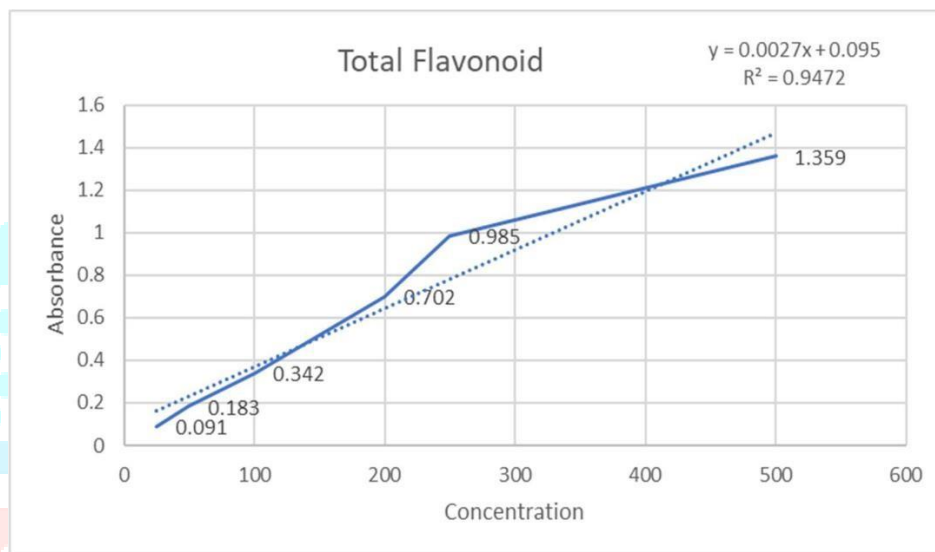
Reduction of Fe^{2+} ion to Fe^{+} was found to rise with increasing concentrations. The standard ascorbic acid showed highest reducing capacity. Among the extracts of the *C.wallichii* showed maximum reducing capacity that is comparable to ascorbic acid.

6.3.2 Total Flavonoid Content

The total flavonoid content of the crude methanol extract of *Actephila excelsa* was determined using 10% AlCl_3 and 1 molar Potassium acetate reagent. The total flavonoid content of the sample was calculated as mg of Quercetin equivalent per gram of dried sample.

Table 8: Absorbance of Gallic acid and the methanol extract of C.wallichii at different concentrations.

Concentrations	Absorbance
500	1.359
250	0.985
200	0.702
100	0.342
50	0.183
25	0.091

**Figure 11: Standard curve of Quercetin for the determination of total flavonoid content****Table 9: Determination of total flavonoid activity of the different extract of C.wallichii**

Concentration	Abs. of sample	m=Wt. of Plant Extract	c	c	V	c*V	A=(c*V)/m
µg/ml		gm	µg/ml	mg/ml	ml	mg	mg/gm
500	0.185	0.0005	3.333	0.0333	1	0.0333	66.67

As shown in table the flavonoid content of methanol extract of C.wallichii was detected 66.67 mg of QE/gm. The absorbance values of the extract of C.wallichii was compared with the standard solution of Quercetin equivalents. The methanolic extract showed significant total flavanoid content that is 66.67 mg of QE/gm.

6.3.3 Evaluation of Antimicrobial Activity

Infectious diseases are the leading cause of death worldwide, accounting for nearly one half of all deaths in tropical countries which are also becoming a significant problem in developed countries. It is calculated that infectious diseases are the underlined causes of death in 8% of the 9 deaths in the united-state. Development of new antimicrobial agents is among the proposed solution to solve this problem. In this regard plants can be provided a good alternative in search for new chemicals with a wide range of antibacterial activities (Casley-Smith, 1997).

Table 10: Antimicrobial screening of methanol extract of C.wallichii

Name of organism	Methanolic extract of C.wallichii (Zone of inhibition)	Azithromycin (30µg/disc)
	mm	
Gram positive		
Staphylococcus aureus	No effect	4.67
Gram negative		
Escherichia coli	No effect	4.1
Vibrio cholerae	No effect	
Salmonella	2.8	24
Fungus		
Aspergillus niger	No effect	-
Sacharomyces cerevisiae	No effect	-

The inhibition of supplied bacterial microbial growth is not well enough for methanol extract of C.wallichii compared to standard Azithromycin. The inhibition area is not satisfied for further study of the extract for new anti-microbial drug.

6.4 Discussion

The present study discusses in vitro antioxidant, antimicrobial activity, of the methanol extract of C.wallichii. After performing the phytochemical screening, the methanol extract of leaf confirmed the presence of carbohydrate, saponins, flavonoids and alkaloids, glycoside, steroid. No trace amount of tannin or terpenoids were present in the extract. The presence of alkaloids, and flavonoids in plant extracts reveal that it can be used as potential immunostimulants. Presences of phytochemicals in the plants protect us from different types of diseases. Flavonoid content can reduce the instances of myocardial infarction and thus the mortality rate up to 25% (Lakshmi et al., 2003). Two third of the plant species contain medicinal values and most of them exerts potential antioxidant activity. Ascorbic acid and also flavonoids are the best exogenous antioxidants which are found in plants (Kasote et al., 2015). The leaf extract possess high antioxidant potentials. The flavonoid content of the methanol extract of C.wallichii was detected 66.67 mg of QE/gm of dried extract. Among the extracts of the C.wallichii showed maximum reducing capacity that is comparable to ascorbic acid. So, it can be clearly understood that for the entire antioxidant analysis performed for the project, the leaf extract showed quite acceptable antioxidant capacity. Therefore, this medicinal plant can be used for preparing antioxidants supplement as it is enriched of potential

antioxidant capacity. The plant shows not well enough anti-microbial activity. Methanol extract *C. wallichii* compare their antibacterial activity for six organisms - *Penicillium*, *Staphylococcus aureus*, *E. coli*, *Saccharomyces cerevisiae* and *Aspergillus niger*, *Salmonella*, *Vibrio cholerae*. The standard drug, *Azithromycin*, showed zone of inhibition against 4 pathogen at 30 gm/ml dose. Thrombolysis is often used as an emergency treatment to dissolve blood clots that form in arteries feeding the heart and brain; the main cause of heart attacks and ischemic strokes and in the arteries of the lungs. A blood clot (thrombus) developed in the circulatory system due to the failure of hemostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as acute myocardial or cerebral infarction, at times leading to death (Gopi et al., 2012). Here different concentration of *C. wallichii* leaves were tested to know its clot lysis percentage compared with standard Streptokinase. Streptokinase and distilled water was taken as positive and negative control respectively. The result of present study indicates, the crude methanolic extract showed mild thrombolytic activity (37.55%). This is only preliminary study and to make final comment, the extract should be thoroughly investigated chemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

Membrane stabilization is a process of maintaining the integrity of biological membranes such as erythrocyte and lysosomal membranes against osmotic and heat-induced lyses (Oyedapo et al., 2010). The erythrocyte resembles lysosomal membranes, as such it has been used as a model system by many workers in the study of interaction of drugs with membranes. The effect of drugs on the stabilization of erythrocytes could be extrapolated to the stabilization of lysosomal membranes (Kumar & Sadique, 1987; Horie et al., 1979; Stubbs et al., 1976). The methanolic extractives of leaves of *C. wallichii* at concentration 10.0 mg/ml were tested to evaluate the activity against lysis of human erythrocyte membrane induced by hypotonic solution, as compared to the standard acetyl salicylic acid (10 mg/ml). In this condition, the samples were found to inhibit lysis of erythrocyte membrane. So, methanolic extract of *C. wallichii* leaves showed significance inhibition (93.41%) hemolysis of RBC as compared to Acetyl salicylic acid (96.28%).

7. Findings and Recommendations

Here are the key findings of the study:

The methanol extract of *C. wallichii* leaves was found to contain the following phytochemicals: carbohydrates, saponins, flavonoids, alkaloids, glycosides, and steroids. However, no traces of tannins or terpenoids were detected in the extract. The presence of alkaloids and flavonoids in the plant extract suggests that it could potentially be used as an immunostimulant. These compounds are known for their immunomodulatory properties and can help protect against various diseases. The study found that the methanol extract of *C. wallichii* leaves possessed high antioxidant potential. The flavonoid content in the extract was determined to be 66.67 mg of quercetin equivalents (QE) per gram of dried extract. The extract showed a significant reducing capacity, comparable to ascorbic acid. This suggests that the plant extract could be used to prepare antioxidant supplements. Unfortunately, the methanol extract of *C. wallichii* leaves did not exhibit strong antimicrobial activity. It was tested against six different

organisms (*Penicillium*, *Staphylococcus aureus*, *E. coli*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Salmonella*, *Vibrio cholerae*), but it did not show significant inhibition compared to the standard antibiotic Azithromycin. Thrombolysis is the process of dissolving blood clots, and it is essential in treating conditions like heart attacks and strokes. The study tested different concentrations of *C. wallichii* leaves for clot lysis compared to the standard drug Streptokinase. The crude methanolic extract exhibited mild thrombolytic activity, with a clot lysis percentage of 37.55%. This suggests that further investigation is needed to explore its potential in thrombolysis. The methanolic extract of *C. wallichii* leaves was tested for its ability to stabilize biological membranes, particularly erythrocyte membranes. It was found to inhibit the lysis of erythrocyte membranes induced by a hypotonic solution, with a significant inhibition rate of 93.41%, compared to the standard drug Acetyl salicylic acid (96.28%).

Based on the findings of the study on the methanol extract of *C. wallichii* leaves, here are some recommendations and potential implications:

The presence of alkaloids and flavonoids in the extract suggests potential immunomodulatory properties. Further research should be conducted to investigate and validate its immunostimulant effects, potentially leading to the development of immunomodulatory therapies or supplements. The high antioxidant potential of the methanol extract, with a flavonoid content comparable to ascorbic acid, suggests that it could be used to formulate antioxidant supplements. Such supplements can contribute to reducing the risk of oxidative stress-related diseases. However, thorough safety and efficacy studies are necessary before commercial production. While the extract did not exhibit strong antimicrobial activity in the initial tests, it might be worthwhile to explore ways to enhance its antimicrobial properties. Researchers could investigate different extraction methods or combination treatments to improve its effectiveness against pathogenic microorganisms. The mild thrombolytic activity observed in the extract is a promising initial finding. Further studies should focus on optimizing the extraction process and exploring the potential mechanisms behind its thrombolytic activity. This could lead to the development of novel thrombolytic agents or treatments. The ability of the extract to inhibit the lysis of erythrocyte membranes is a positive result. Research could continue to understand the specific components responsible for this effect and explore its potential applications in protecting biological membranes from damage. The study acknowledges that these findings are preliminary and recommend further comprehensive chemical and pharmacological investigations. This includes isolating and identifying specific bioactive compounds responsible for the observed effects and conducting more extensive in vitro and in vivo studies to assess safety and efficacy.

7. Conclusion

In the developed countries, the use of medicinal plants or herbal medicines has extended widely in the last half of the twentieth century. That is why the interest of conducting research on medicinal plants is increasing day by day. For this project, the medicinal plant *C.wallichii* was preferred to discover some constructive properties such as antioxidant, membrane stabilizing and mild antimicrobial and thrombolytic activity. After conducting the research, it can be decided that the leaf extract of *C.wallichii* confirmed different biological activities such as antioxidant, membrane stabilizing as well as presence of some important phytochemicals. This plant extract can be used in reducing the oxidative stress.its also have significant Antidiarrhoeal activity Group of different essential evaluations such as analgesic activity and cytotoxic activity is further need to be performed by using this plant. If it is possible, it can proposed from our study, for further research we can isolate different molecule from the plant to find out and characterize the secondary metabolites of the plant and correlate the present finding of the current study. If it is possible we can experiment in such a manner that, the isolated compound exhibit the desired result from the current study, may be approached for new drug development process. Study can keep crucial role in modern medicine development.

References

- Ahmed, T., Noor, F. A., Soma, M. A., Aktar, F., & Kuddus, M. R. (2019). Leaves of *Clerodendrum wallichii* Merr. Exhibit analgesic, antioxidant and cytotoxic activities. *Bangladesh Pharmaceutical Journal*, 22(2), 208–213.
- Bainsal, N., Bora, K. S., & Singh, J. (2022). Pharmacognostic Evaluation and Phytochemical Screening of an Unexplored Herb: *Thalictrum Foliolosum*dc. *Biomedical and Pharmacology Journal*, 15(2), 985–991.
- Gopi, G., Elumalai, A., & Jayasri, P. (2012). A concise review on *Tagetes erecta*. *International Journal of Phytopharmacy Research*, 3(1), 16–19.
- Horie, T., De Wit, C. de, Goudriaan, J., & Bensink, J. (1979). A formal template for the development of cucumber in its vegetative stage (I, II and III). *Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen. Serie C: Biological and Medical Sciences*, 82(4), 433–479.
- Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences*, 11(8), 982.
- Kifayatullah, M., Mustafa, M. S., Sengupta, P., Sarker, M. M. R., Das, A., & Das, S. K. (2015). Evaluation of the acute and sub-acute toxicity of the ethanolic extract of *Pericampylus glaucus* (Lam.) Merr. In BALB/c mice. *Journal of Acute Disease*, 4(4), 309–315.
- Kumar, P., & Sadique, J. (1987). The biochemical mode of action of *Gynandropsis gynandra* in inflammation. *Fitoterapia*, 58(6), 379–386.
- Lakshmi, V., Pandey, K., Puri, A., Saxena, R., & Saxena, K. (2003). Immunostimulant principles from *Curculigo orchoides*. *Journal of Ethnopharmacology*, 89(2–3), 181–184.

- Oyedapo, O., Akinpelu, B., Akinwunmi, K., Adeyinka, M., & Sipeolu, F. (2010). Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. *International Journal of Plant Physiology and Biochemistry*, 2(4), 46–51.
- Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. *International Journal of Herbal Medicine*, 4(4), 59–64.
- Shrivastava, N., & Patel, T. (2007). *Clerodendrum* and healthcare: An overview. *Medicinal and Aromatic Plant Science and Biotechnology*, 1(1), 142–150.
- Stubbs, G. W., Litman, B. J., & Barenholz, Y. (1976). Microviscosity of the hydrocarbon region of the bovine retinal rod outer segment disk membrane determined by fluorescent probe measurements. *Biochemistry*, 15(13), 2766–2772.

