Original Research Article

In-Vitro Pharmacological Investigation of *Ludwigiaadscendens*

ABSTRACT

Aims: This research objective was to investigate some in-vitro properties of methanol-extracted plant extracts of *Ludwigiaadscendens* (the water primrose). The primary method of assessment for the methanolic extract of *Ludwigiaadscendens* (MELA) was investigated for phytochemical screening. It was determined to look into the plant's potential for in vitro activities, such as antioxidant, antimicrobial, anti-inflammatory anti-arthritis, cytotoxic, thrombolytic and membrane stabilizing activity due to the pharmaceutical interest in its component parts.

Place and Duration of Study: This research was carried out between November 2022 to January 2023 at the Laboratory of Phytochemistry and Pharmacology in the Department of Pharmacy and Laboratory of Microbiology, Stamford University Bangladesh, Dhaka.

Methodology: Phytochemical screening was performed utilizing a battery of test reagents. Antioxidant, antimicrobial, anti-inflammatory, anti-arthritic, cytotoxic, thrombolytic and membrane stabilizing activities were measured at various doses using the DPPH Free Radical Scavenging Assay, the Disk Diffusion Method, Egg Albumin Protein Denaturation Assay, the Inhibition of Protein Denaturation Assay, the Brine Shrimp Lethality Bioassay, Clot lysis assay and heat induced and hypotonic induced haemolysis method.

Results: The findings of research on MELA's phytochemical screening showed the presence of a wide range of various phytochemical components. The higher antioxidant activity of MEPLA is shown by its IC_{50} value of 85.76 µg/mL. Significant antibacterial action was shown by MELA. As compared to the standard ascorbic acid, this extract showed significantly greater anti-arthritic and anti-inflammatory effects. MELA exhibited much higher LC_{50} value which is 1.147 µg/mL than the typical vincristine-sulphate in a lethality test with brine shrimp. Comparatively speaking, this herb has quite powerful thrombolytic action. Significant membrane stabilizing action has also been shown by MELA

Conclusion: To wrap it up, it is clear that this plants phytochemical can be used for wide range of drug discovery field due to its potent pharmacological actions.

Keywords: Antioxidant, antimicrobial, anti-inflammatory, antiarthritic, cytotoxic, thrombolysis.

1. INTRODUCTION

Humans have long relied on medicinal plants as a reliable source of both therapeutic and preventative medical treatment preparations, and many essential bioactive chemicals have been extracted from these plants. Especially in developing nations, it is believed that as much as 80% of the global population relies on ethnomedicine and products on a daily basis.

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Sick patients in poor countries often use both Western and alternative treatments. In the poor, rural areas of developing nations, where only traditional medicine exists, its use is widespread because of its low cost and high accessibility [1].

Drug formulations that are based on antioxidants are used for the prevention and treatment of complicated illnesses such as stroke, atherosclerosis, cancer, diabetes, and Alzheimer's disease [2].

The -diphenyl-picrylhydrazyl (DPPH) free radical scavenging technique is the first method that may be used to evaluate the antioxidant capacity of a chemical, an extract, or other biological sources [3].This method is used until now as an ideal method for determining antioxidant activity.

In the realm of scientific research, one of the most important goals is to locate novel antibacterial substances that are able to combat bacteria that have developed resistance to existing antibiotics [4].

Most phytochemicals have significant medicinal properties, including insecticidal, , spasmolytic, antibacterial, antifungal, anti-constipative, anti-plasmodial, and antioxidant properties, among others [5]. Methods like Disc diffusion test, MIC test are used for determining antibacterial activity.

Blood artery dilation in response to an irritant allows fluid and cells to enter the interstices that define inflammation. Inflammation causes redness, heat, malignancy, discomfort, and dysfunction. Anti-inflammatory medications are useful for treating these disorders medically [6]. Egg albumin is a well-known protein which is used in protein denaturation assay for detecting anti-inflammatory effect.

Arthritis is an autoimmune disease that causes pain, swelling, and stiffness in the joints. Conventional treatments for rheumatoid arthritis, such as non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatoid drugs (DMARDs), and corticosteroids, have the goals of reducing the patient's pain and joint inflammation, minimizing loss of function, and slowing the progression of joint damage [7].

The brine shrimp lethality test for larvae (napulii) has been used as a bioassay for a variety of hazardous compounds, including an antitumor pre-screening test. This makes it easier to isolate biologically active compounds for use in the formulation of medicines [8].

In the modern world, thrombosis is recognized as one of the three primary factors that contribute to the morbidity and death associated with cardiovascular disease. In layman's terms, thrombosis refers to a localized clotting of the blood, which may occur either inside the arterial or the venous circulation and has severe repercussions for one's health [9].

There are around 82 different species of flowering plants that belong to the genus *Ludwigia L*., which belongs to the family Onagraceae and is extensively dispersed in both South and North America [3].

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The plant can grow up to 50cm in height and has bright green, lanceolate leaves that are arranged oppositely along the stem. The leaves can vary in size and shape depending on the conditions in which the plant is grown. *Ludwigiaadscendens* is an adaptable plant that can grow in a range of water conditions, from pH 6.0 to 7.8 and soft to hard water. It requires high light levels and adequate nutrients to grow well, and does best with a nutrient-rich substrate and CO_2 injection.

There are a number of traditional applications for *Ludwigia* species, including anti-inflammatory, antidiarrheal, anti-diabetic and antibacterial symptom alleviating properties. Flavonoids, saponins, phenolic compounds, and triterpenes are only some of the many types of phytochemicals that have been attributed to the Genus *Ludwigia's abundance*[10]. Quercetin, quercetin 3-O-rhamnoside or quercitrin, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-rutinoside, kaempferol 3-O-glucoside, myricetin 3-O-rhamnoside or myricitrin, and myricetin 3-O-galactoside have all been identified as being present in *Ludwigiaadscendens*[11].

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This research was carried out to detect its antioxidant, antimicrobial, anti-inflammatory, antiarthritic, cytotoxic, thrombolysis and membrane stabilizing activity.

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2. MATERIAL AND METHODS

2.1 Plant material

The sample plant *Ludwigiaadscendens*was taken in October 2022 from West-Delpara, Kutubpur, Narayanganj, Dhaka. The experts at the Bangladesh National Herbarium in Mirpur, which is located in Dhaka, were able to accurately identify the plant (Accession number: DACB 87895). After accession of the plant, the whole plant was dried in shade for 11 days and crushed into fine powder for doing experiments.

2.2 Reagents

Sigma Chemical Co., USA provided methanol, NaOH, diluted HCl acid, concentrated H_2SO_4, and acetic acid. It was through Orion Infusion Ltd. that we acquired the sterile saline solution. Diazepam and diclofenac sodium injections were produced by Square Pharmaceuticals Ltd. The DMSO came from the German business Merck. Bovine Serum Albumin was purchased from Polysciences, Inc. India. Streptokinase was brought from Incepta Pharmaceuticals Ltd, Bangladesh.

2.3 Preparation of plant extract

The *Ludwigiaadscendens* whole fresh plant was obtained by removing the soil. The whole plant was then washed in water that was at room temperature in order to completely eliminate the dust. *Ludwigiaadscendens* were washed, then air dried for 12 to 15 days in the shade. After the plant had properly dried, the whole thing was broken up into tiny bits and combined by a machine. 66g of powdered *Ludwigiaadscendens* were soaked in up to three fingers of methanol for three days. The maceration process was periodically helped by stirring during this time. After three days, the extract was filtered using filter paper. 6.79 g of extract were generated by air drying the solvent out. The raw extract was maintained in a beaker, kept cool, and shielded from the light.

2.4 Phytochemical screening

The phytochemical analysis was carried out by following the protocols that were outlined in the prior research [12]. It was determined whether or not samples of the methanolic extract of *Ludwigiaadscendens* contained the following phyto constituents: alkaloids, steroids, saponins, reducing sugar, flavonoids, carbohydrates, tannins, gums, glycosides, and phenols.

2.5 Antioxidant activity

2.5.1 DPPH radical scavenging activity

The antioxidant activity of the samples was evaluated using the technique provided by Naz and Bano [13] This evaluation was conducted on the basis of the samples' ability to scavenge the stable DPPH free radical. The following concentrations of the sample were used: 500, 250, 125, 62.5, 31.25, 15.62, and 7. µg/mL). 1mL of each of these concentrations was added to 3 mL of a 0.1 mmol/L MeOH solution of DPPH. After 30 minutes of observation in the dark, absorbance at 517 nm was measured, and the percentage of inhibitory activity was computed using the formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of blank} - \text{Absorbance of Test sample})}{\text{Absorbance of blank}} \times 100$$

2.6 Antimicrobial test

2.6.1 Test Microorganisms

5 bacteria named *Bacillus cereus, Klebsiella pneumoniae, Vibrio cholerae, Escherichia coli, Streptococcus aureus* and 4 fungi named *Penicilliumchrysogenum, Aspergillusniger, Yeast budding* and*Mucorhiemalis*was collected from laboratory of microbiology, Stamford University Bangladesh.

2.6.2 Antimicrobial susceptibility test

Microorganisms were placed into Mueller Hinton Agar (MHA) added petri dishes, and then sterilized discs (6 mm in diameter) were inserted into the agar plates as part of the disc diffusion procedure [14]. MEPA was dissolved in the appropriate volumes of solvents to produce solutions of known concentration (300, 500 and 700 µg/mL). The petri dishes were then incubated at 4 °C for 2 hours to allow the extracts to diffuse into the agar. Finally, the inhibition zones surrounding the discs were recorded after incubating the petri dishes at 37 °C for 1 day. After 24 hours, the zone of inhibition was measured in cm.

2.7In vitro anti-inflammatory assay

This method was used with minor modification which was mentioned in previous research [15]. 0.2 mL of egg albumin (from a fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4), and 2 mL of MELA at concentrations of 31, 62.5, 125, 250, 500, and 1000 µg/mL were mixed to produce the reaction mixture (5 mL). Controls consisted of a same volume of double-distilled water. After this, the solutions were heated to 70° C for 5 minutes and incubated at 37°C for 15 minutes in a BOD incubator (Labline Technologies). Once they had cooled, we assessed their absorbance at 660 nm (SHIMADZU, UV 1800) with a vehicle blank and their viscosity using an Ostwald viscometer. While determining absorbance and viscosity, a reference medication of diclofenac sodium at final concentrations of (31, 62.5, 125, 250, 500, and 1000 µg/mL) was utilized. To determine the proportion of protein denaturation that was prevented, the following formula was used:

$$\% \text{ of Inhibition} = \frac{Abs\ of\ control - Abs\ of\ test\ sample}{Abs\ of\ control} \times 100$$

Here, Abs= Absorbance

2.8 In vitro anti-arthritis assay

The reaction mixture consisted of 0.5 mL of 5% aqueous solution of bovine serum albumin (BSA) and 0.05 ml of different concentrations (31, 62.5, 125, 250, 500, and 1000 µg/mL) of the MELA. The pH of the reaction mixture was adjusted to 6.3 using 1 N HCl and it was then incubated at 37 °C for 20 min and maintained at 57 °C for 30 min. The reaction mixture was allowed to cool, then 2.5 mL of phosphate buffer saline was added. Using spectrophotometer, the turbidity was measured at 660 nm. The standard was 1 mg/ml of diclofenac sodium, while the control included 0.05 mL of pure water [16]. The formula for determining the degree to which protein denaturation was prevented was as follows:

% of Inhibition = [100 - (OD of test solution - OD of product control) x100]

In which optical density (OD) is the descriptor.

2.9 Cytotoxic assay

2.9.1 Brine shrimp lethality assay

The methanolic crude extract cytotoxic activity at different concentrations were predicted using the brine shrimp lethality assays [17]. For the experiment, 4 mg of each extract was dissolved in dimethyl sulfoxide (DMSO), and solutions of different concentrations were prepared using the serial dilution approach with simulated seawater (400, 200, 100, 50, 12,5, 6,25, 3,13, 1.56, 0.78 µg/mL). The indicated vials holding 10 nauplii of live brine shrimp in 5 ml of artificial seawater were then dosed with the solutions. Following 24 hours, the test tubes were examined using a magnifying lens to count the number of surviving nauplii. Using these results, we were able to determine the percent lethality of brine shrimp nauplii at each concentration and as a control. As a reference standard, vincristine sulphate was employed. The LC_{50} was calculated using a logarithmic plot of concentration against death rate.

2.10 Thrombolytic activity assay

M.M. Miah et al.'s approach [18] was used to assess the plant fractions' thrombolytic activity. Blood was drawn from healthy volunteers and then split into vials of 5 mL using pre-weighed sterile vials containing 1 mL each. The clotting time for blood samples was 45 minutes at 37°C. When the produced serum was drained from the vials, a new weight measurement was taken to establish the clot weight. The vials were then filled with an aqueous solution of the plant fractions (2 mg/mL) with a total volume of 100 µL. A negative non-thrombolytic control (100 µL) and a standard (streptokinase, 30,000 I.U.) were used. Next, after 90 minutes, we put everything in an incubator at 37°C. The clot's released fluid was drained and the vials' new weights were recorded after incubation. The percentage of clot lysis used to quantify the thrombolytic activity was determined using the following equation.

$$\% \text{ of Clot lysis} = \frac{A}{B} \times 100$$

where A and B reflect, respectively, the weight of the released clot before treatment and after treatment.

2.11 Membrane stabilizing activity

2.11.1 Preparation of Human Red Blood Cells (HRBC) Suspension

A sterile Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water) was combined with an equivalent amount of freshly drawn human blood. Packed cells were washed three times with iso-saline (0.85%, pH 7.2), and the blood was centrifuged at 3000 rpm for 10 minutes. The volume of the blood was measured and reconstituted as 10% v/v suspension with iso-saline [19].

2.11.2 Heat Induced Haemolysis

The stability of the human red blood cell membrane via hypotonicity-induced membrane lysis is the foundational principle at play here. The assay mixture includes 1 mL of phosphate buffer with a pH of 7.4 and a concentration of 0.15 M, 2 mL of hypo saline with a concentration of 0.36%, 0.5 ml of HRBC suspension with a volume-to-volume ratio of 10%, and 0.5 mL of plant extracts. This mixture was incubated at 37°C for 30 minutes with a control containing distilled water instead of hypo saline in order to A spectrophotometer set to 560 nm was used to provide an estimate of the amount of haemoglobin present in the suspension. The following formula may be used to determine the percentage of haemolysis of the HRBC membrane:

The percentage of haemolysis may be calculated as follows:

$$\% \text{ Haemolysis} = \frac{Optical\ \ density\ \ of\ Test\ sample}{Optical\ \ density\ of\ \ Control} \times 100$$

The percentage of HRBC membrane stabilisation can be calculated as follows:

% Protection = 100 – [$\frac{Optical\ \ density\ \ of\ Test\ sample}{Optical\ \ density\ \ of\ Control}$ × 100]

2.11 Statistical analysis

All bioassay readings were conducted in triplicate, and the tabular data is provided as the mean standard deviation. Statistical analyses were conducted using Excel.

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening

MELA was subjected to a phytochemical analysis, which determined that it has a variety of phytochemicals, such as tannin, flavonoids, saponin, reducing sugars, alkaloids, gums, glycosides, steroids, and phenolics, among others. Yet, there were no carbs to be found in **Table 1**.

Table 1. Results of phytochemical screening

Phytochemical constituent	MESAL
Flavonoid	+++
Tannin	+
Alkaloid	+
Steroid	+++
Saponin	+++
Carbohydrate	-
Glycoside	++
Reducing Sugar	++
Gum	++
Phenolics	+++

Here (+++) indicates a higher amount, (++) indicates a moderate amount, (+) indicates mild amount, and (–) indicates absence

Phytochemical analysis of plant extracts showed that certain phytoconstituents were present while steroids were absent in extract (Table 1). The result indicated that the plants contain quite a lot of chemical elements, which may be responsible for the many pharmacological activities. Despite the fact that this research did not look into their precise functions. Several scientists have confirmed that the plant contains phytochemicals [11] .

3.2 Antioxidant activity

A violet solution in methanol is generated by the free radical DPPH, which is stable at room temperature. In the presence of an antioxidant molecule, it is reduced, resulting in colourless methanol solutions. The use of DPPH is a simple and quick method for assessing antioxidants. Table 2. demonstrates the DPPH radical scavenging properties of the plant's whole extract and ascorbic acid. With an IC_{50} value of 85.76 µg/mL, MELA demonstrated the highest level of DPPH radical scavenging activity. All of the samples demonstrated dose-dependent activity for DPPH radical scavenging.

Table 2. *In vitro* free radical scavenging effect of MELA

Samples	Concentrations	% Inhibition	IC_{50} in DPPH radical scavenging analysis (µg/mL)
Ascorbic Acid	7.81	55.72	38.31
	15.625	59.67	
	31.25	70.57	
	62.5	73.70	
	125	81.60	
	250	88.55	
	500	93.86	
MELA	7.81	57.09	85.76
	15.625	78.48	
	31.25	86.62	
	62.5	90.11	
	125	94.76	
	250	97.09	
	500	98.83	

According to previous research, natural products have been shown to contain a wide range of antioxidants [20]. So, it is possible that like other plants, this plant also may have higher antioxidant potential. This extract could be abundant in antioxidants like flavonoids, which neutralize free radicals. Further research on the chemical constituents of such extracts, as well as research using other models, such as lipid peroxidation and in vivo testing, are required to describe them as biological antioxidants.

3.3 Antimicrobial Assays

By measuring the zone of growth inhibition in mm, the antibacterial and antifungal activity of different dosages of plant extract was examined against gram-positive and gram-negative bacteria as well as four distinct types of fungi (mm). The results showed that when plant extract concentration increased, the inhibitory zone grew (Table 6, 7). Zone of inhibition for antibacterial activity was 08-18 where zone of inhibition of fungi was 06-14 mm. The following results demonstrates that MELA have highest antibacterial activity against *Escherichia coli* and *Bacillus cereus* which causes hazardous diseases like urinary tract infections, respiratory illness and pneumonia gastrointestinal illness, diarrhoea etc.

Table 3. Inhibition zone of MELA against different bacteria

Diameter of Zone of Inhibition (mm)				
Test organisms	MELA (300 µg/disc)	MELA (500 µg/disc)	MELA (700 µg/disc)	Ciprofloxacin
Gram Positive Bacteria				
Bacillus cereus	14	15	18	25
Staphylococcus aureus	12	14	15	26
Gram Negative Bacteria				
Escherichia coli	13	16	18	25
Vibrio cholerae	12	13	14	27
Klebsiella pneumonia	08	09	11	24

Table 4. Inhibition zone of MELA against different fungi

Diameter of Zone of Inhibition (mm)				
Test organisms	MELA (300 µg/disc)	MELA (500 µg/disc)	MELA (700 µg/disc)	Griseofulvin (50 µg/disk)
Fungi				
Penicilliumchrysogenum	06	10	14	19
Aspergillusniger	08	09	10	20
Yeast budding	10	11	14	21
Mucorhiemalis	07	10	13	21

Antibacterial and antifungal tests are essential to assess the efficacy of antimicrobial agents. Antibacterial tests are performed to determine the ability of a substance to inhibit the growth of bacteria, whereas antifungal tests are conducted to evaluate the potential of a substance to suppress the growth of fungi. One of the most common methods of testing for antibacterial activity is the agar diffusion method or Kirby-Bauer method. In this test, bacteria are grown on an agar plate, and disks containing the antimicrobial agent are placed on the surface of the agar. The size of the zone of inhibition around each disk indicates the degree of inhibition of bacterial growth. The diameter of the zone of inhibition is measured and compared with a standard chart to determine the antimicrobial susceptibility of the bacteria.
Antifungal tests are also performed using the agar diffusion method, which is similar to the Kirby-Bauer method used for bacterial tests. In the agar diffusion method, a fungal strain is grown on an agar medium, and disks containing the antifungal agent are placed on the

surface of the agar. The zone of inhibition around each disk is measured to determine the antifungal activity of the substance.

To wrap it up, antibacterial and antifungal tests are crucial to assess the efficacy of antimicrobial agents. The agar diffusion and broth dilution methods are commonly used to determine the degree of inhibition or kill rate of bacteria and fungi by different substances. Such tests are essential in the development and evaluation of new antimicrobial agents, as well as in the selection of appropriate agents for the treatment of bacterial and fungal infections.

3.4 Anti-inflammatory effect

An essential technique for evaluating the potential therapeutic benefits of different plants is the in vitro anti-inflammatory test of plant extracts. The egg albumin denaturation test is often used to assess the anti-inflammatory effects of plant extracts. Table. 5 illustrates that MEPLL have highest egg albumin denaturation effect on the concentration of 1000 and 500 µg/mL compared to the standard Acetyl salicylic acid, a standard drug which is often used as anti-inflammatory medication.

Table 5. Percentage inhibition in egg albumin denaturation of MEPLA

Samples	Concentrations	% of inhibition µg/mL
Acetyl salicylic acid	62.5	20
	125	33.33
	250	66.67
	500	77.78
	1000	88.88
MEPLL	62.5	77.37
	125	88.28
	250	90.98
	500	91.89
	1000	93.69

The egg albumin denaturation assay is based on the principle that the denaturation of egg albumin is a good indicator of inflammatory reactions. Inflammation leads to the production of various inflammatory mediators, such as prostaglandins and cytokines, which cause the denaturation of egg albumin. Thus, if a plant extract can inhibit the denaturation of egg albumin, it can be considered as having anti-inflammatory properties. The results obtained from the egg albumin denaturation assay can be used to screen plant extracts for anti-inflammatory activities. However, it is important to note that this assay is not suitable for all plant extracts, as some may interfere with the egg albumin matrix, causing false-positive or false-negative results. In conclusion, the egg albumin denaturation assay is a simple and

efficient method for evaluating the anti-inflammatory effects of plant extracts. However, it should be used in conjunction with other assays to confirm the anti-inflammatory properties of plant extracts. In this research during phytochemical screening, tannins, flavonoids, terpenoids, phenols, steroids, alkaloids and saponins were found which are may be responsible for such significant effect [21].

3.5 Anti-arthritics activity

Table 6 displays the impact on protein denaturation inhibition. Results showed that both MELA and diclofenac-sodium displayed a concentration-dependent inhibition of protein denaturation over the whole tested concentration range (62.5-1000 µg/mL). The protein denaturation inhibitory activity of the crude extract was 98.195 at 1000 µg/mL, which was comparable to the activity of Diclofenac Sodium (94.59% at 1000 µg/mL).

Table 6. Percentage inhibition in protein denaturation of MELA

Samples	Concentrations	% of inhibition
Diclofenac Sodium	62.5	84.64
	125	87.38
	250	89.18
	500	93.69
	1000	94.59
MELA	62.5	71.17
	125	78.37
	250	88.28
	500	90.99
	1000	98.19

The anti-arthritic activity of plant extracts is often investigated in vitro by denaturing Bovine serum albumin (BSA). To do this, researchers examine the impact of herbal extracts on the stability of bovine serum albumin (BSA), a protein with many structural and functional similarities to human serum albumin (HSA). Protein denaturation due to thermal or chemical treatment, or exposure to extremes in pH or ionic strength, is measured in this assay. An anti-inflammatory drug or an anti-arthritic chemical may lessen or prevent denaturation from occurring. The denaturation mechanism involves shifts in electrostatic, hydrogen, hydrophobic, and disulphide bonding [22]. Phytoconstituents including flavonoids, alkaloids, terpenoids, and others are responsible for the anti-denaturation action of an herbal extract [23]. These chemicals have been studied for their wide range of biological effects, which include antioxidant, anti-inflammatory, and immunomodulatory properties. It is well recognized how these plant extracts work to alleviate arthritis symptoms. Arthritis is an inflammatory joint disease that has several contributing causes, including genetics, the environment, and the immune system. Contributing components include the production of inflammatory cytokines, free radicals, and reactive oxygen species (ROS). By blocking inflammatory pathways including NF-kB, COX-2, and TNF-, scavenging free radicals, and modulating the immune system, the herbal extracts display anti-inflammatory, antioxidant, and immunomodulatory effects. The suppression of the inflammatory cascade is one of the

primary mechanisms via which herbal extracts exert their anti-arthritic activity. Arthritis symptoms including swelling, redness, and discomfort are all mitigated by this. These plant extracts help keep the joint tissue healthy and prevent additional injury by reducing inflammation. Finally, screening for anti-inflammatory and anti-arthritic potential of herbal extracts may be facilitated using the in vitro anti-arthritic test of plant extract by denaturation of Bovine serum albumin. The bioactive elements of these plant extracts are responsible for their anti-arthritic activities via inhibiting inflammatory pathways, scavenging free radicals, and modulating the immune system, respectively.

3.7 Cytotoxic assay

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The brine shrimp lethality assay is a widely used bioassay for the preliminary screening of plant extracts for their biological activity. This test is used for the evaluation of toxicity of compounds and to assess the cytotoxic properties of plant extracts. Cytotoxicity testing as a first step in screening plants gives important information about the plant's extract's potential antitumor and anticancer properties. The MELA's cytotoxic impact on the growth of brine shrimp was evaluated.

Table 7.Result of brine shrimp bio-assay and LC_{50} value of MELA

Sample name	Concentrations	Mortality %	LC_{50} value
Vincristine Sulphate	7.81	40	20.57
	15.625	40	
	31.25	50	
	62.5	60	
	125	100	
	250	100	
	500	100	
MELA	0.98	20	1.147
	1.95	30	
	3.91	50	
	7.81	60	
	15.625	70	
	31.25	80	
	62.5	80	
	125	90	
	250	90	
	500	100	

The in vitro brine shrimp lethality assay is based on the principle that brine shrimp nauplii are highly sensitive to toxic compounds. The extract is mixed with a saline solution containing brine shrimp nauplii, and the mortality of the nauplii is observed and recorded after 24 hours of exposure. The concentration of the extract that kills 50% of the brine shrimp nauplii is considered as the LC_{50} value, which is used as an indicator of the bioactivity of the plant extract. The mechanism of action of plant extract in the brine shrimp lethality assay is not well understood. However, it is believed that the toxic compounds in the extract bind to the membrane of the brine shrimp nauplii and damage the cells, leading to cell death. The toxicity may be due to the presence of alkaloids, flavonoids, and other bioactive compounds in the extract, which have been shown to possess cytotoxic properties [24].

Using a brine shrimp lethality assay, the cytotoxic impact of the MELA methanol extract was assessed. 500>250>125>62.5>31.25>15.125>7.81>3.91>1.95>0.98 µg/mL was the order of cytotoxicity. The findings indicated that the MELA had an inverse relationship with brine shrimp survival. The current finding is consistent with previous reports that MELA shown 100% cytotoxicity at high dosage for brine shrimp. The many bioactive chemicals found in this plant like polyphenols, flavonoids, tannins etc may thus be the cause of their ethno-pharmacological actions.

3.8 ~~Results of t~~Thrombolysis

The potential of plant extracts to dissolve blood clots is evaluated using an~~in vitro~~ thrombolysis test, which is performed in a controlled laboratory setting. When the body is injured, blood cells and proteins clump together to create a clot to prevent further bleeding. However, excessive clotting may cause potentially fatal illnesses including heart attacks and strokes. In the following table demonstrates that the percent of clot lysis of MELA is very significant compared to the standard streptokinase.

Table 8. Mean value of percent of clot lysis (N=15)

Sample	% of clot lysis
Negative control	7.296
Streptokinase	91.304
MELA	87.17

In the ~~in vitro~~ thrombolysis test, a clot is generally incubated in a test tube with a plant extract, and the quantity of clot that dissolves over time is then measured. The weight of the clot or the quantity of fibrinolytic activity produced by the disintegration of the clot may be used to measure the degree of thrombolysis. It is believed that plant extracts' capacity to stimulate the body's fibrinolytic system plays a role in how they work to break up clots. The fibrinolytic system is an intricate network of enzymes and proteins that cooperate to dissolve clots and stop new clotting. Plant extracts may increase these fibrinolytic enzymes' activity and hasten the clot's disintegration. Also, certain plant extracts may prevent the platelet aggregation process, which is what causes blood cells and proteins to adhere together to form clots, in order to prevent the development of clots. The in vitro thrombolysis test of plant extract, in conclusion, is a useful technique for evaluating the potential of plant-based medicines for the treatment of thrombotic diseases. The augmentation of fibrinolytic activity and the inhibition of platelet aggregation are both components of the intricate and multidimensional process by which plant extracts induce thrombolysis. To develop efficient therapeutics for thrombotic disorders and to better understand the mechanisms of action of these extracts, further research is required.

~~3.9~~3.8 Membrane stabilizing activity

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The in vitro membrane stabilizing test is a popular technique for evaluating the medical properties of plants. This experiment assesses how well plant extracts can protect against membrane lysis and damage, two prominent side effects of pathological situations including inflammation and oxidative stress. To perform the test, osmotic stress is applied to a lipid membrane, and the amount to which the membrane stabilizes in the presence of the plant extract is then measured. In Table 9. It portrays that MELA have significant membrane stabilizing activity which is 63.14% compared to the standard drug.

Table 9. MELA haemolysis of erythrocyte membrane

Sample	% of hemolysis	% of protection
Diclofenac-sodium	26.36	73.64
MELA	36.86	63.14

The membrane stabilizing effect of plant extract is attributed to the presence of secondary metabolites, such as flavonoids, alkaloids, and phenolic compounds, which possess anti-inflammatory, antioxidant and free radical scavenging properties. These bioactive compounds interact with the lipid bilayer of the membrane, preventing the influx of ions and maintaining the integrity of the cell membrane [25]. The mechanism of the membrane stabilizing effect of plant extract involves multiple pathways. Firstly, the plant extract inhibits the release of inflammatory mediators, such as prostaglandins, leukotrienes and histamine, by interfering with the arachidonic acid metabolism. Secondly, it modulates the activity of enzymes such as cyclooxygenase and lipoxygenase, which are responsible for the production of these mediators. Thirdly, the extract reduces the generation of free radicals and reactive oxygen species, which cause oxidative damage to the lipid membrane. The antioxidant activity of the extract helps to maintain the membrane fluidity and elasticity. Furthermore, the extract may also inhibit the activation of transcription factors, such as nuclear factor kappa B (NF-κB), which play a crucial role in the transcription of pro-inflammatory and pro-apoptotic genes. By downregulating the expression of these genes, the extract suppresses the inflammatory response and prevents apoptosis, ultimately leading to membrane stabilization. In conclusion, the in vitro membrane stabilizing test is a useful method to screen the potential therapeutic value of plant extracts. The membrane stabilizing effect is predominantly attributed to the presence of various bioactive compounds present in the extract, which exert anti-inflammatory, antioxidant and free radical scavenging activities. The mechanism of such effect involves multiple pathways, including the inhibition of inflammatory mediators, modulation of enzyme activity, reduction of free radicals, and down regulation of pro-inflammatory genes.

4. CONCLUSION

Ludwigiaadscendens is a native blooming plant to Bangladesh, as well as many other nations. It is clear from the aforementioned description that *Ludwigiaadscendens* contains significant phytochemicals and has a range of biological functions. The results of the current study have confirmed that the plant *Ludwigiaadscendens'*s crude methanolic extract exhibited potential antioxidant, antimicrobial, anti-inflammatory, antiarthritic, cytotoxic, thrombolysis, and membrane properties. This implies that *Ludwigiaadscendens*can be useful in the development of new drugs. The plant is thus a suitable choice for more systematic, chemical, and biological tests to identify the active component.

Comment [NMFH8]: *invitro*

Comment [NMFH9]: The plant extract should be tested *in vivo*in lab animals for a real assessment

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication of this research report.

ETHICAL APPROVAL

This research followed all rules set forth by the US Food and Drug Administration, the Declaration of Helsinki, and the International Conference on Harmonization. Stamford University Bangladesh's Faculty of Science examined and accepted the research procedure and written consent form (reference number: SUB/ERC/202301). Everyone who took part in the study had to submit a documented consent form, and they had the right to withdraw at any moment.

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