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EXPLORING THE THERAPEUTIC POTENTIAL OF ELEUSINE INDICA PLANT: PROMISING ANTIOXIDANT AND ANALGESIC ACTIVITY WITH LACK OF ANTIMICROBIAL AND THROMBOLYTIC ACTIVITY

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ABSTRACT
Background: *Eleusine indica* is used as a traditional medicine in Bangladesh. It is significantly valued for its wound-healing properties and is often used as an anthelmintic and pain reliever in rural areas. **Objective:** This study examines the pharmacological effects of *Eleusine indica* methanolic extracts, highlighting antioxidant, antimicrobial, thrombolytic, and analgesic properties in animal models. Also, contribute to future research directions. **Methods:** The antioxidant properties were determined using the DPPH free radical scavenging assay, with ascorbic acid as the positive control, and the inhibition percentage was calculated. Antimicrobial activity was determined using the disc diffusion method. The thrombolytic Potential was determined through in vitro clot lysis assays. However, the % of clot lysis was used to gauge the thrombolytic effects. For assessing analgesic activity, Swiss albino mice were utilized; analgesic efficacy was evaluated by calculating the percentage inhibition against abdominal writhing. **Results:** The free radical scavenging assay demonstrated with an IC_{50} of 43.67 µg/ml, comparable to ascorbic acid's IC_{50} of 36.22 µg/ml. However, the methanolic extract showed no antimicrobial activity. In thrombolytic assays, the extract induced 26.79% clot lysis. With a 55.57% reduction in writhing, the extract group (500 mg/kg) exhibited significant analgesic activity, approaching the standard group's 73% inhibition. **Conclusion:** The Outcome of the study shows *Eleusine indica* has potential antioxidant and analgesic activity, with a lack of antimicrobial and thrombolytic properties. The results support the application of traditional medicine. highlighting the importance of antioxidant and analgesic activity, in future investigations aimed at isolating and identifying the specific compounds driving its pharmacological activities.

INTRODUCTION

Medicinal plants have been a part of traditional healthcare practices for centuries and are still widely used today. It's the most vital healthcare source for many humans. The WHO (World Health Organization) calculated that more than 75% of people widely use herbal drugs for their regular primary

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healthcare needs. *Eleusine indica* is the botanical name of a medicinal plant locally known as Jharua, a species of grass belonging to the family Poaceae. It is a small annual grass distributed throughout the warmer areas of the world to about 50 degrees latitude. It is especially used in local areas for stomach problems and urine retention; it has long been used in traditional Thai medicine because of its diuretic, anti-inflammatory, and antipyretic effects. An annual *Eleusine indica*, 30-60 cm tall, tufted, slightly compressed, and leaves distichous, flat or folded, as long as the stem, linear. It contains cyanogenetic glucoside, triglochinin, ochratoxin A, and α-amylase inhibitors. The seed coat contains phenolic compounds and flavonoids. Grains take on albuminoids, starch, and fatty oil, orientin, isoorientin, vitexin, isovitexin, saponarin, violanthin, lucenin-1, and tricin are found in leaves.

Eleusine indica (L.) Gaertn., commonly known as goosegrass, is an annual diploid grass species (2n = 18) believed to originate from Africa and Asia. Over the years, numerous herbicides have been utilized to manage E. indica across various crops. However, since the 1970s, herbicide-resistant biotypes with resistance to multiple Sites of Action (SoA) have been documented globally. Glyphosate is the most important and widely used herbicide in world agriculture. The repeated use of dinitroaniline herbicides on the southern United States cotton and soybean fields has resulted in resistant biotypes of one of the world's worst weeds, *Eleusine indica*. The whole plant, mainly the root, is used for its diuretic, anti-helminthic, and febrifuge effects and for treating coughs and various ailments. The seeds are sometimes used as famine food, particularly in the northern areas of Bangladesh.

Progressively, there is a strong focus on research to identify plants that have the potential to be antioxidant, antimicrobial, thrombolytic, and analgesic, so they can be utilized to treat a wide range of injuries and prevent disease. Antioxidants protect cells from damage caused by free radicals, unstable molecules produced during oxidation in normal metabolic processes. When free radicals or other reactive oxygen species (ROS) accumulate at high levels, they can lead to oxidative stress, which can cause both direct and indirect harm to the body. Oxygen mediates chemical reactions that metabolize fats, proteins, and carbohydrates to convert them into energy. Some free radicals can attack the healthy cells of the body. These are responsible for damage, disease, and severe disorders. The production and subsequent role of free radicals in various diseases, including myocardial ischemia, cancer development, liver damage, inflammatory conditions, cataract formation, and Alzheimer's disease, are well-established. Determining the plants or natural sources with antioxidant properties could be a useful approach for developing antioxidant-based medications. Many natural antioxidants, including fruits, vegetables, herbs, and spices, come from plant sources. The primary natural antioxidants found in plant materials are carotenoids (xanthophylls and carotenes), vitamins (E and C), and polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes). These natural antioxidants, particularly polyphenols and carotenoids, generally have various biological effects, including anti-inflammatory, antibacterial, and anticancer properties. A few studies have found that E. indica extract is capable of lowering the stable DPPH level in a dose-dependent manner.

Plants are considered as one of the most promising sources for new antimicrobial discovery. Since ancient times, Plant materials have been used in traditional medicine to treat various microbial diseases. The word antimicrobial was derived from the Greek words anti (against), mikros (little), and bios (life), and means all agents that act against microbial organisms. The word “antimicrobials” refers to all agents that play against all types of microorganisms – bacteria (antibacterial), viruses (antiviral), fungi (antifungal), and protozoa (antiprotozoal). Plant secondary metabolites are mostly responsible for their antimicrobial activity. Principal phytochemicals with antimicrobial characteristics are phenolics and polyphenols (flavonoids, quinones, tannins, coumarins), terpenoids, alkaloids, lectins, and polypeptides. Previous research indicates that *E. indica*'s methanolic extract has a notable phenolic content, while its ethyl acetate extract exhibits antibacterial properties. The current study aims to determine the same activity in the methanolic extract.

Numerous plant species have shown in vitro thrombolytic activity in various scientific studies. Thrombolysis, also known as thrombolytic therapy, is a treatment to dissolve excessive clots in blood vessels, improve blood flow, and prevent damage to tissues and organs. Suppose a blockage or blood clot is considered to be life-threatening. In that case, thrombolysis may be an option to be induced intravenously as soon as possible after the onset of the symptoms of a heart attack, stroke, or pulmonary embolism. Foods and herbs with anti-thrombotic effects have been shown in experiments to lower the risk of thrombosis.

Thus, determining *E. indica*'s thrombolytic effect was interesting because no published research has been done to support this effect.

Compared to the modern medical sciences in developing nations, the traditional practice of using plants as analgesic drugs in folk medicine is much older. Natural products that have long been used to treat pain include the opium poppy (*Papaver somniferum*) and the bark of the willow tree (*Salix spp*.). Later active compounds that have been isolated from these two plants to provide analgesic effects are opium and salicin. Analgesics are a class of drugs that selectively relieve pain without interfering with nerve impulse conduction, significantly altering sensory perception, or affecting consciousness. They are divided into two categories: anti-inflammatory drugs, which reduce pain by decreasing local inflammation, and opioids, which exert their effects on the brain. The analgesic properties of herbal extracts have been discovered through various pain assessment methods, including the formalin test, hot-plate test, writhing test, light tail-flick test, and tail immersion test. A thorough investigation of Bangladeshi species of *E. indica* is necessary to determine their analgesic potential because there have been limited studies on the analgesic properties of the plant (only the Nigerian plant).
The study aimed to ascertain a baseline for using *Eleusine indica* for various medical purposes, such as antioxidant, antimicrobial, thrombolytic, and analgesic effects. Additionally, this study may act as a springboard for further investigation and identification of the specific compounds accountable for the plant's diverse pharmacological characteristics.

MATERIAL AND METHODS

Collection of plant material and preparation of extract

In June 2017, *Eluesine indica* (Poaceae) was collected from a district of Bangladesh named Jessore. The plant was identified and authenticated by the CABI Digital Library. The entire plant of *Eleusine indica* was carefully cleaned to remove any foreign material, air-dried at room temperature for 5 days, and then sun-dried in the shade for an additional 5 days. Afterward, the plant was ground into a fine powder. The powder was mixed with methanol (80 g of powder in 2 L of methanol) and left to stir occasionally for 3 days. The extract was filtered twice, first through cotton wool and subsequently through Whatman filter paper (No. 1). Finally, the filtrate was evaporated to dryness using a rotary evaporator (Lab Tech). This produced a residue that constituted the crude methanolic extract of *Eleusine indica* (MEEI). The extraction yield was calculated, and the crude extract was kept at +4°C until further use. The plant extract was preserved at the same temperature for 3 months.

Figure 1: *Eleusine indica* plant

Drugs, chemicals and reagents

Ciprofloxacin, Diclofenac Na, Acetic Acid, Ascorbic acid. 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Methanol, and distilled water were used in the study.

Experimental animal

Swiss albino mice of both sexes, weighing 18 and 30 grams, were used in this study. The mice were sourced from Jahangirnagar University, Bangladesh, and given three days to acclimatize before the experiments commenced. The animals were housed in a polypropylene cage at a well-ventilated, hygienic animal house under constant room temperature. They were fed mice chow prepared according to the formula developed by the Bangladesh Council of Scientific and Industrial Research (BCSIR). All experiments on mice were carried out in absolute compliance with the ethical guide for care and use of laboratory animals approved by the Ethical Review Committee, Faculty of Allied Health Science, Daffodil International University (ID: DIU/FAHS/REC16/ethical_ clearance/04/2023-26). The standard method of euthanasia was also used for the termination of mice.

DPPH free radical scavenging activity for antioxidant property

Stock solutions (10 mg/ml) of the plant extracts were prepared in ethanol, from which serial dilutions were carried out to obtain concentrations of 500, 250, 125, 62.5, 31.96, 15.625, 7.183, 3.901, 1.90 & 0.97g/ml diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was

measured at 517 nm, and the corresponding inhibition percentage was calculated from these values. The percentage inhibition was then plotted against the log concentration, and IC50 was determined from the graph. The experiment was duplicated, and each concentration's average absorbance was recorded. Ascorbic acid was used as a positive control. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

$$\%\ \boldsymbol{of\ inhibition} = \frac{\boldsymbol{A_b - A_a}}{\boldsymbol{A_b}} \times \mathbf{100}$$

Where A_b is the absorbance of the control (without test samples), and A_a is the absorbance of the test samples. Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$$\boldsymbol{I}\ \% = \frac{\mathbf{1} - \boldsymbol{A_{sample}}}{\boldsymbol{A_{blank}}} \times \mathbf{100}$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).

The extract concentration resulting in 50% inhibition (IC50) was calculated from the graph plotting the inhibition percentage against the extract concentration. Ascorbic acid was used as a positive control. Tests were carried out in triplicate, and the average value was taken.

Antimicrobial activity by the disc diffusion method

The antimicrobial activity of the plant extract was determined by the method of Bauer et al. (1966) with slight modification. In this method, the measured amount of the test samples is dissolved in definite volumes of solvent to give solutions of known concentration (µg/ml). Then, sterile material filter paper discs are impregnated with a known amount of test substances using a micropipette and dried. Standard antibiotic discs and discs on which the solvent used to dissolve the samples is adsorbed and dried are used as a positive and negative control, respectively. These discs are then placed in Petri dishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using a sterile transfer loop for antimicrobial screening. The plates are then kept at 40°C to facilitate maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates are then kept in an incubator (37°C) for 12-18 hours to allow the growth of the microorganisms.

If the test material has any antimicrobial activity, it will inhibit the growth of microorganisms, giving a clear, distinct zone called the “zone of inhibition”. The antibacterial activity of the test agent was assessed by measuring the diameter of the inhibition zone in millimeters. The experiments were conducted three times, and the average value was recorded.

Evaluation of analgesic activity

Collection of animals: This study used Swiss albino mice (18-30g weight) collected from Jahangirnagar University, Bangladesh.

Environment control: They are housed in standard polypropylene cages and kept under controlled room temperature (24 ±2°C) and relative humidity (60-70%) in a 12-hour light-dark cycle. They are fed ICDDR, B-formulated rodent food and water, as these animals are very sensitive to environmental changes.

Acetic Acid writhing reflex method: The Koster method was modified by Danbisya and Lee to conduct this study. Twenty-four albino mice of both sexes were randomly selected and then divided into four groups (A-D) of six animals each. Animals were fasted for 12 hours and later treated as follows: Group A mice were given tween 20 solution 10 ml/kg (negative control group), group B mice were given 15mg/kg diclofenac Na (positive control group) while groups C, D received 250, 500 mg/kg methanolic extract of *Eluesine indica* respectively all by gastric gavage.

All mice received intraperitoneal (i.p.) injections of 0.7% glacial acetic acid (10 ml/kg) one hour after the drug and extract administration to cause pain manifested as writhing or constriction of the abdomen. Every mouse's number of writhes was calculated and recorded for 30 minutes.

$$\frac{\boldsymbol{Mean\ control - \ Mean\ treated\ group}}{\boldsymbol{Mean\ of\ the\ control\ group}} \times \mathbf{100}$$

The degree of analgesia was estimated by calculating the percentage protection against abdominal writhing using the formula mentioned above.

Thrombolytic activity evaluation

Preparation of extract dose: Extract concentration, Stock solution = 100mg/10ml**.** Standard: Streptokinase 1500000 IU/5ml, Dose: 30000 IU in 100µl

Procedure: *In vitro* clot lysis activity of the leaves was performed using Streptokinase as a positive control and water as a negative control according to the reference method with minor

modifications. Ethical guidelines were observed, and aseptic precautions were taken. Five milliliters of venous blood was drawn from healthy volunteers (n = 3) with no history of smoking, lipid-lowering medications, oral contraceptives, or anticoagulant therapy, and transferred into different pre-weighed sterile micro-centrifuge tubes (1 ml per tube). The micro-centrifuge tubes were then incubated at 37°C for 45 minutes. After clot formation, serum was completely removed from the tubes (carried out without disturbing the clot formed), and each tube with a clot was again weighed to determine the weight of the clot (clot weight = weight of clot-containing tube – weight of tube alone).

To each micro-centrifuge tube containing a pre-weighed clot, 100 µl of solutions with different extracts at a 1 mg/mL concentration were added. For the positive control, 100 µl of streptokinase was added, and 100 µl of sterilized distilled water was added to separate control tubes for the negative non-thrombolytic control. All tubes were incubated at 37°C for 90 minutes and monitored for clot lysis. After incubation, the fluid was removed from the tubes, and the tubes were reweighed to determine the change in weight after clot disruption. The difference in weight was then calculated, and the result was expressed as the percentage of clot lysis using the below equation..

$$\textbf{\textit{Percentage of clot lysis}} = \frac{\textbf{\textit{weight of lysis clot}}}{\textbf{\textit{initial clot weight}}} \times \mathbf{100}$$

RESULT & DISCUSSION

DPPH free radical scavenging activity

The DPPH test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. Practically, the reaction brings about the reduction of DPPH radicals to the corresponding hydrazine, which is manifested by a color change from violet to yellow, which is monitored spectrophotometrically. It is evident from the table that the % scavenging of DPPH radical was found to rise with increasing concentration of the samples. The IC_{50} value of the positive control ascorbic acid has been found 36.22µg/ml whereas the methanol extract of the plant resulting IC_{50} value 43.67µg/ml (See Table 1). The methanol extract showed promising DPPH free radical scavenging activity with an IC_{50} value of 43.67µg/ml compared to the positive control ascorbic acid with an IC_{50} value of 36.22µg/ml (Figure 4). Research indicates that the phenolic components of *E. indica*, along with the presence of additional secondary metabolites like glucosides and C-glycosylflavone, are responsible for its antioxidant activity. Therefore, the plant may be a potential source of antioxidant activity.

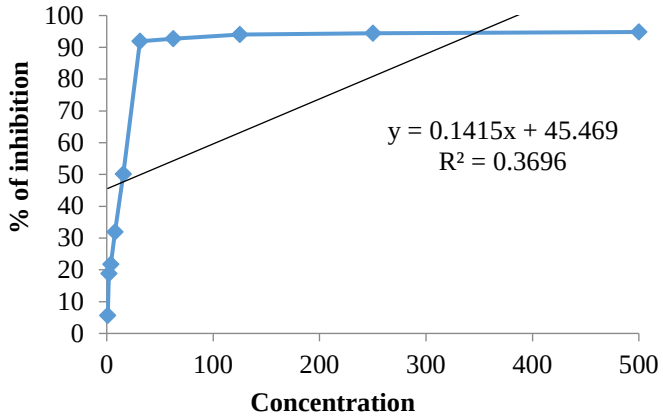

Figure 2: Free radical scavenging activity in relation to increasing concentration of ascorbic acid

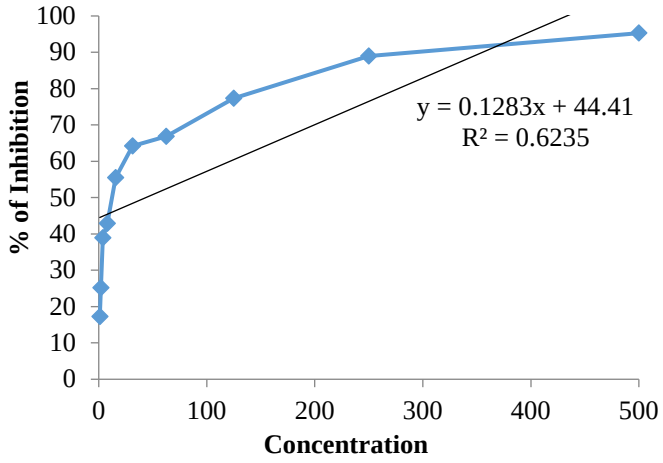

Figure 3: Free radical scavenging activity of methanolic extract of *Eleusine indica.*

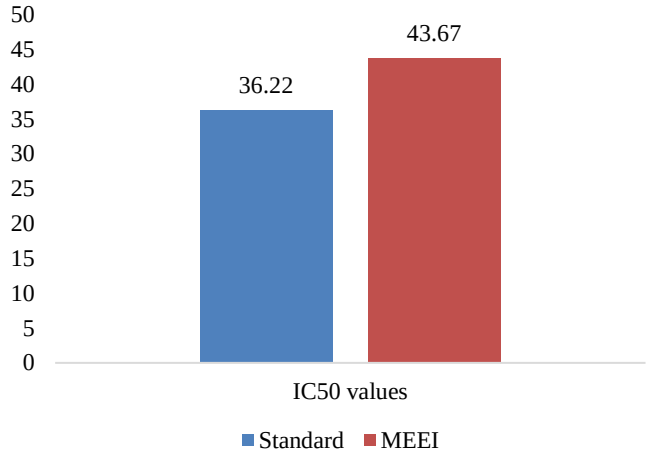

Figure 4: Comparison of inhibition concentrations (IC50) between standard and sample

Table 1: Comparing the inhibition of oxidation potentials between ascorbic acid and methanolic extract of the plant

Absorbance of control	Conc. (µg/ml)	Ascorbic acid			Methanolic extract of *Eleusine indica* (MEEI)		
		Absorbance	Inhibition (%)	IC_{50} (µg/ml)	Absorbance	Inhibition (%)	IC_{50} (µg/ml)
0.483	500	0.025	94.82	36.22	0.0228	95.27	43.67
	250	0.027	94.40		0.054	88.92	
	125	125	93.99		0.109	77.37	
	62.5	62.5	92.75		0.160	66.82	
	31.25	31.25	91.93		0.173	64.2	
	15.625	15.625	50.10		0.215	55.49	
	7.813	7.813	31.88		0.276	42.86	
	3.906	3.906	21.74		0.295	38.92	
	1.953	1.953	18.84		0.631	25.19	
	0.977	0.977	5.59		0.400	17.25	

The methanol extract showed promising DPPH free radical scavenging activity with an IC_{50} value of 43.67µg/ml compared to the positive control ascorbic acid with an IC_{50} value of 36.22µg/ml (Fig-4). Research indicates that the phenolic components of *E. indica*, along with additional secondary metabolites like glucosides and C-glycosylflavone, are responsible for its antioxidant activity. Therefore, the plant may be a potential source of antioxidant activity.

Antimicrobial Test

Both the standard ciprofloxacin and the methanolic extract of the plant were tested for antimicrobial activity. The standard antimicrobial agent demonstrated inhibition against *Bacillus subtilis* and *Vibrio metschnikovii*, while the plant extract showed no inhibition against the tested microbial strains. Methanolic extract of the plant may not be active against these selected bacterial strains (see Table 2). A similar result was found in another article, where methanolic extract showed no inhibitory effect on all tested bacterial strains. However, the ethanolic extract exhibited a broad-spectrum antibacterial activity against all tested bacteria except *Bacillus subtilis*.

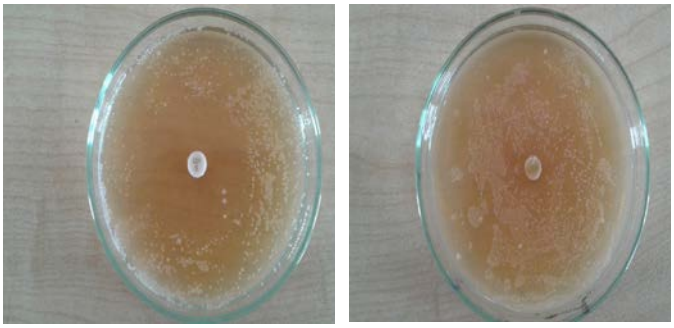

Figure 5: Microbial inhibition by the disc diffusion method

Table 2: Comparison of in vitro antibacterial activity of standard (Ciprofloxacin) and methanolic extract of the plant

Bacterial strains	Diameter of zone of inhibition (mm)	
	Ciprofloxacin (50µg/disc)	MEEI (50µg/disc)
Bacillus subtilis (Gram Positive)	28	No Inhibition
Vibrio metschnikovii (Gram Negative)	20	No Inhibition
Klebsiella oxytoca (Gram Negative)	----	No Inhibition

Thrombolytic activity

The thrombolytic activity of the plant extract was ascertained by comparing the propensity of clot lysis of the MEEI to standard Streptokinase. When 100 µl of streptokinase (30,000 I.U.) was used against the thrombus, the percentage of clot lysis was 87.58%, but in the case of the extract, the percentage of clot lysis was minimal. (26.79%). Therefore, it can be said that the crude methanolic extract of *Eleusine indica* showed the least amount of clot lysis activity (see Table 3).

Analgesic activity

The *Eleusine indica* significantly ($p<0.05$) inhibited writhing in several doses (see Table 4). A higher dose (500 mg/kg) of *Eleusine indica* inhibited 55.57 % of writhing compared to the standard drug Diclofenac Na, which inhibited 73 % of writhing. Here, strongly noticeable effects were observed with the extract group (500 mg/kg), and this effect is similar to that of the standard group (see Table 4).

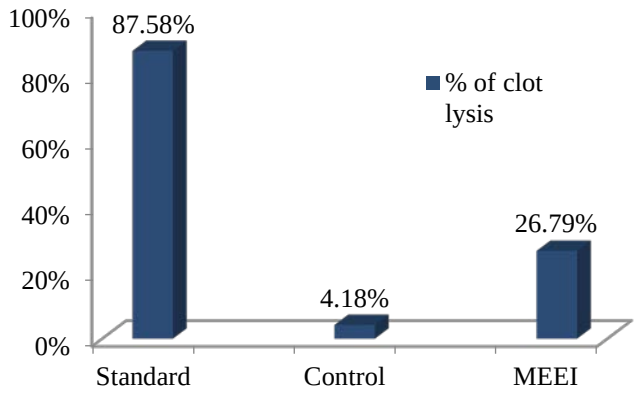

Figure 6: Capabilities of clot lysis by plant extracts in comparison to the other two groups

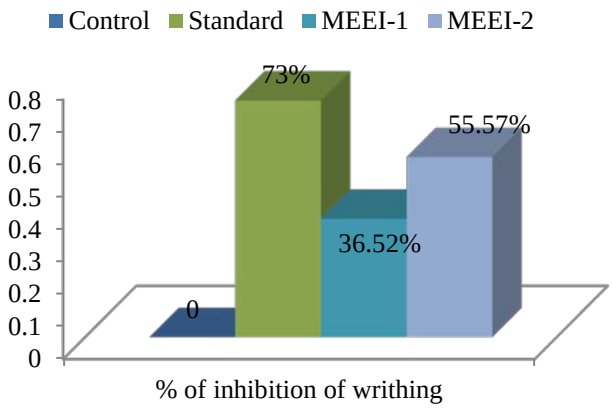

Figure 7: Comparison of analgesic activity (inhibition of writhing) of *E. indica* with standard and control groups

Table 3: Comparative view of the percent of clot lysis activity among the three groups

Sample	1st clot + tube weight (gm)	1st clot weight (gm)	2nd clot + tube weight (gm)	2nd clot weight (gm)	% of Lysis
Standard (Streptokinase)	1.652	0.822	0.9321	0.1021	87.58
Control (Distil water)	1.452	0.622	1.4261	0.596	4.18
MEEI	1.39	0.56	1.24	0.41	26.79

Table 4: Inhibitory effect of *Eleusine indica* in comparison to the standard and control groups on acetic acid-induced writhing reflex in mice

Group	Treatment mg/kg	No. of mice	No. of writhing	Mean ± SME of writhing	% of inhibition
Control	Tween 20 solution	1	25	21±2.08	--
		2	20		
		3	18		
Standard	Diclofenac Na 75mg/kg	1	4	5.67±1.20	73%
		2	5		
		3	8		
MEEI-1	EI 250 mg/kg	1	15	13.33±0.88	36.52%
		2	12		
		3	13		
MEEI-2	EI 500 mg/kg	1	7	9.33±1.20	55.57%
		2	10		
		3	11		

Therefore, it is evident that *E. indica* has dose-dependent analgesic effects. Several studies strongly suggest that the ability to inhibit acetic acid–induced writhing of this extract may in part be linked to its inhibition of lipoxygenase and/or cyclooxygenase in peripheral tissues, reducing PGE2 synthesis and interfering with the mechanism of transduction in primary afferent nociceptor [25]. Based on the summarized results, it can be concluded that the plant extract demonstrates potential antioxidant and analgesic activity while showing no antimicrobial and minor thrombolytic effects. However, there are several limitations to consider. For instance, the activity may vary depending on the sample size, choice of solvents, and the need for more comprehensive isolation of active compounds to enhance transparency. Further investigation is required to identify which specific plant part is responsible for the observed bioactivity.

CONCLUSION

For thousands of years, plants worldwide have been used for medicinal purposes, and many studies have been conducted to

validate these traditional uses scientifically. *Eleusine indica* is one such significant medicinal plant. This study aimed to explore and evaluate *Eleusine indica* for its antioxidant, antimicrobial, analgesic, and thrombolytic properties. It aimed to provide a valuable lead for future research, based on a combined approach of exploitation and exploration. The findings suggest that the plant holds a promising source of potential antioxidant, analgesic, and mild anticoagulant activities, which could be beneficial in treating human ailments.

Moreover, the thrombolytic and antimicrobial properties should be further studied on a larger scale to validate their activity. However, further research with larger sample sizes is required to detect significant variations. Advanced analytical methods can be used in future research studies to identify the precise component and part of the plant that contributes to its therapeutic effects. HPLC, GC-MS, or LC-MS are powerful analytical techniques that will help to identify specific pharmaceutically active compounds in plants by compound profiling. These also supports measuring the concentration of bioactive compounds and structure elucidation. The results from this study support the use of *Eleusine indica* in medicinal health and nutraceutical applications due to its antioxidant and analgesic properties. Nevertheless, the exact mechanisms underlying it's therapeutic effects are not yet fully understood and need further studies. Overall, *Eleusine indica* could be a valuable source of promising therapeutics.

ETHICAL STATEMENTS

The handling and use of the animals were in accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals. The handling and use of the experimental animals were approved by the Animal Ethics Committee of the Daffodil International University (ID- DIU/FAHS/REC-116/Ethical clearance/04/2023-26).

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FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Farhana Israt Jahan took the lead in writing the manuscript and contributed to conceptualization, data curation, formal analysis, investigation, and supervision. Asif Hossain Anik and Sumaiya Akter conducted formal analysis and contributed to the investigation and methodology. All authors contributed to the manuscript's writing, review, and editing. Additionally, all authors provided critical feedback and helped shape the research, analysis, and manuscript.

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