South Asian Research Journal of Natural Products

Volume 6, Issue 3, Page 271-282, 2023; Article no.SARJNP.109897

Biological Investigation of *In-vitro* Anti-Inflammatory, Antifungal, Anti-Arthritic, Thrombolytic, Membrane Stabilizing an *In-vivo* Acute Toxicological Activity of *Launaea asplenifolia*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/109897

Original Research Article

Received: 09/10/2023
Accepted: 12/12/2023
Published: 22/12/2023

ABSTRACT

The present research is designed for evaluating the in-vitro activities of the ethanolic extract of *Launaea asplenifolia* (EELA), specifically its anti-inflammatory, antifungal, anti-arthritic, thrombolytic, membrane-stabilizing properties and acute toxicological activity. The present study aimed to conduct a primary evaluation of EELA through phytochemical screening. The screening revealed the presence of various secondary metabolites, including alkaloids, flavonoids, saponins, glycosides, carbohydrates, gums, and reducing sugars. These compounds are known to have

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diverse biological activities and are of interest to researchers in the fields of medicine. This research also investigated the anti-arthritic and anti-inflammatory therapeutic properties of EELA using in-vitro methods of protein denaturation. The results indicates that EELA had a significant impact on the reduction of arthritis and inflammation. The extracts demonstrated a percentage inhibition of 95.49% and 87.05% at a concentration of 1000 µg/mL, which were very significant compared to the standard diclofenac sodium (94.59%) and acetyl salicylic acid (88.88%) at the same concentration. This research investigated the potential antifungal properties of EELA using Disk Diffusion Method. The results of the research indicate that EELA exhibited antifungal activity against various types of fungi. The zone of inhibition ranged from 06-13 mm, depending on the specific type of fungi tested. The membrane-stabilizing properties of EELA using the heat-induced hemolysis method. The results indicated that EELA exhibited a protective effect against hemolysis, with a percent protection value of 82.02%. This value was compared to that of the standard diclofenac sodium, value of 73.63%. The thrombolytic activity of EELA was evaluated using the clot lysis method. The results indicated a positive correlation between the amount of extract used and the ability to break up blood clots. Notably, a value of 93.36% was obtained, which is statistically significant when compared to the standard streptokinase value of 91.304%. The in-vivo acute toxicological activity test was done using Cinnamon Oil Induced Method which showed 100% mortality rate at the dose of 5000mg/kg which was very potent compared to the cinnamon oil. In conclusion, the phytochemical present in the plant exhibits significant pharmacological effects, which may get used for drug discovery applications.

Keywords: Anti-arthritic; antifungal; anti-inflammatory; membrane stabilizing and Launaea asplenifolia.

1. INTRODUCTION

Phytochemicals and nutraceuticals found in plants are increasingly being investigated to investigate their possible therapeutic properties [1]. Micronutrients and secondary metabolites may be found in significant amounts in wild plant species. The importance of these varied species in maintaining human and environmental health has been brought to light, with the primary focus being on sustainable development and the safety of food supplies across the worldwide [2] [3]. The use of antibiotics to treat illnesses has become standard practice in the contemporary era, but antibiotic resistance has emerged as one of the greatest obstacles facing scientists and medical professionals today. When bacterial and fungal diseases weren't treated properly, thousands of people perished. A rising global problem, the creation of resistant organisms is a direct result of the widespread use of antibiotics [4]. Infectious diseases in humans are often caused by pathogenic fungus, and they are notoriously difficult to cure [5]. Both *Trichophyton mentagrophytes* and *Microsporum gypseum* are considered to be among the most substantial pathogens that may cause life-threatening infections. Herbal extracts are often used as a therapeutic option for a variety of fungus-related diseases [6]. The mechanism that is associated with tissue damage and in which a variety of chemical mediators are generated is referred to as inflammation [7]. These mediators have a significant correlation with a number of illnesses, including autoimmune and cardiovascular conditions, cancer, arthritis, diabetes, and others [8]. One putative mechanism of action for the anti-inflammatory effect is a stabilization of membranes. The after effects of inflammation may be treated with any number of available anti-inflammatory medications. Studies have looked at the influence of several medications, including herbal preparations, on the stability of erythrocyte membranes during hypotonic and heat stress. Intestinal side effects and mucosal erosions that may evolve into ulcers have been linked to these medicines in a number of studies [9]. Herbal medicine involves utilization of a wide variety of plants and plant parts, including but not limited to: seeds, berries, roots, leaves, fruits, bark, flowers, and even the whole plant [10].

Launaea asplenifolia is a species of plant that is considered to be a weed and is a member of the Asteraceae family (sunflower family). Typically, it is grown in Asian nations such as Bangladesh, India, Pakistan, and Sri Lanka, amongst others. Throughout the months of September to June, it blooms in damp and shaded regions, as well as along the riverbank [11]. This plant may often be found growing in rice fields in the countries of South and South-East Asia. It is a herbaceous plant that has extensive and perennial subsurface parts (roots and root-stocks), yet its above-ground parts only last for a short period of

time. This plant is an annual herb that may grow 12 to 25 cm in height. Both as a vegetable and as an ingredient in ayurveda medicine, the leaves of the plant are used. Leukoderma is an affliction of the skin that may be remedied with the help of this herb [12]. This particular water extract has an antifungal action against a fungus known as *Rhizoma solani* [13].

In the current research, the organic soluble components of a methanol extract of the complete plant were examined for the first time for their phytochemical screening, in-vitro anti-inflammatory, anti-arthritic, antifungal, membrane stabilizing and thrombolytic activity. These activities were investigated for the whole plant as a whole.

2. MATERIALS AND METHODS

2.1 Plant Material

The whole *Launaea asplenifolia* plant that was gathered in May of 2022 in Madaripur, Bangladesh. Shahrina, Chief Scientific Officer of the Bangladesh National Herbarium in Mirpur, Dhaka, Bangladesh, was brought in to identify the specimens. It has been documented by depositing a voucher specimen (DACB: 71498) in the Herbarium for future research.

2.2 Reagents

Sigma Chemical Co., USA provided methanol, NaOH, diluted HCl acid, concentrated H_2SO_4. The sterile saline solution was purchased from Orion Infusion Ltd. Diclofenac sodium was produced by Square Pharmaceuticals Ltd. Bovine Serum Albumin was purchased from Polysciences, Inc. India. Streptokinase was purchased from Incepta Pharmaceuticals Ltd, Bangladesh.

2.3 Plant Extract Preparation

After two weeks of drying at room temperature, the plant components were ready to be used. Powder was made from the dried plant components, which were then pulverized, sieved, and stored in plastic containers until required. It took 24 hours of percolating a 1171g sample of powdered dried entire plant in 1100mL of 80% ethanol+20% distilled water. Then, a Millipore filter was used to transfer the extract to a conical flask. Using a rotary evaporator, the resulting extracts were concentrated and dried.

2.4 Phytochemical Screening

The chemical constituents of plants are known to yield various therapeutic properties [14]. The qualitative screening of freshly prepared EELA was conducted to determine the presence of various phytochemicals, including but not limited to alkaloids, carbohydrates, saponins, glycosides, reducing sugar, flavonoids, tannins, and steroids.

2.5 *In vitro* Anti-Inflammatory Test

According to the previous research, in preparation for the experiment, a range of concentrations (62.5, 125, 250, 500, and 1000 µg/mL) were utilized to create a mixture. The mixture consisted of a total volume of 5mL of the reaction mixture, which included 2.8mL of phosphate buffered saline (PBS) with a pH of 6.4, 0.2mL of egg albumin derived from a hen's egg, and 2mL of EELA. The control group was administered with equimolar amounts of double-distilled water. The mixtures were subjected to a temperature of 70°C for a duration of 5 minutes following an incubation period of 15 minutes at a temperature of (37±2) °C, utilizing a Biological Oxygen Demand (BOD) incubator. Following the cooling process, the absorbance of the mixtures was measured at a wavelength of 660nm [15]. As a point of comparison, Acetyl Salicylic acid is also utilized at an equivalent concentration as a standard. Fractional equation for calculating percentage of inhibition of protein denaturation:

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

2.6 Anti-Arthritic Activity Test

The present study employed the "inhibition of protein denaturation" [7] assay to evaluate the potential anti-arthritic activity of EELA extracts in vitro, with diclofenac sodium being employed as the reference standard. A solution comprising 5% bovine serum albumin in water was used in conjunction with 0.05 mL of the test solution to obtain a total solution volume of 0.5 mL, which included EELA extracts. The test control solution comprises a 0.5 mL volume, with the remaining 0.05 mL consisting of a 5% w/v aqueous solution of bovine serum albumin and distilled water. The control sample comprises 0.5 mL of a solution consisting of 95% distilled water and 5% of the test solution. The standard solution comprises of a 5% w/v aqueous solution of bovine serum albumin and 0.05 mL of diclofenac sodium, with a total volume of 0.5 mL. Samples of diclofenac

sodium considered the standard of reference, as well as EELA extracts, were collected at concentrations of 62.5, 125, 250, 500, and 1000 µg/mL. The pH of all solutions was reduced to 6.3 by utilizing a 1 N HCl solution. Following a 20-minute incubation period at a temperature of 37°C, the temperature was subsequently elevated to 57°C and maintained at this level for 3 minutes. Upon cooling the solutions, 2.5 mL of phosphate buffer was introduced. The absorbance at a wavelength of 416 nm was determined using a UV-Visible spectrophotometer. Under controlled conditions, proteins undergo complete denaturation. In this study, diclofenac sodium was employed as a comparator. Table 3 presents a summary of the extent to which inhibition of protein denaturation occurs at different doses. Formula for determining protein denaturation inhibition percentage:

$$\% \text{ Inhibition} = \frac{(\text{OD of Control} - \text{OD of sample})}{\text{OD of control}} \times 100$$

Here, OD means optical density.

2.7 Antifungal Susceptibility Test

Fungal strains: From Microbiology Department of Stamford University Bangladesh and Bangladesh Council of Scientific and Industrial Research, pure culture of fungi (*Penicillium chrysogenum*, *Aspergillus niger*, *Mucor hiemalis* and Yeast budding) was obtained.

Disc diffusion method: The investigation of the antifungal properties of EELA was conducted through the utilisation of the disc diffusion assay, as outlined by Klančnik et al. (2010). The present methodology involved the formation of a solid agar medium within a Petri dish. Subsequently, a uniform distribution of 1mL of culture for each fungus was applied on the medium. A 6mm diameter sterile filter paper disc was utilized to apply 10µL of diluted EELA onto the surface of each agar plate. The experiment involved the administration of EELA at varying concentrations (300, 500, 700µg/mL). Subsequently, the plates were placed onto the incubator for a duration of 24 hours. A disc containing Griseofulvin was utilized as an antifungal agent for the positive control, whereas a disc containing methanol was utilized for the negative control. Antifungal activity was determined after a 24-hour period by measuring the size of the inhibition zone surrounding the disc in millimeters, as reported by Singh, Zaman, and Gupta in 2007.

2.8 *In vitro* Thrombolytic Test

Blood sample: A medical professional facilitated the collection of 4mL of venous blood from a sample of 10 healthy human volunteers who had no history of consuming blood thinners, nicotine, or oral contraceptives. The entire procedure obtained ethical clearance from the Institutional Ethics Council of Stamford University Bangladesh. Subsequently, a quantity of 500µL of fresh blood was dispensed into a set of ten micro centrifuge tubes.

Affirmation of Donors consent: Each donor was provided with a consent form that outlined the objectives of the research, the title of the project, and the quantity of blood that would be collected. The present study's depiction encompasses the investigation of the potential consumption of therapy by volunteers, the occurrence of any form of irritation to the piercing area, and the duration of the blood collection process.

Clot lysis method: The present experiment employed a previously published research paper by [16] to determine the percentage of clot lysis. The present study involved the collection of 2.5mL of fresh blood, which was subsequently distributed into 10 pre-weighed sterile microcentrifuge tubes, each containing 0.5mL of blood. The tubes were then subjected to incubation at 37°C for a period of 45 minutes. Following the incubation period, the serum was intentionally removed from the tubes in a manner that did not disrupt the clot. In order to determine the weight of the clot, the tubes were subjected to a second weighing procedure. The clot weight was calculated by subtracting the weight of the tube without the clot from the weight of the tube containing the clot (Clot weight = weight of clot-containing tube - weight of tube without clot). The experimental procedure involved the addition of 100µL of EELA to each microcentrifuge tube containing a pre-weighed clot. The lyophilized streptokinase vial was reconstituted by adding 2.5mL of phosphate-buffered saline (PBS) and subsequently mixed thoroughly. A positive control was established by filling a volume of 100µL of the suspension into the tube. A negative control was implemented in the study by utilizing 100µL of distilled water. The assessment of clot lysis was conducted in each tube subsequent to incubation at a temperature of 37° for a duration of 90 minutes. Following the incubation period, the tubes were subjected to a second weighing in order to assess any

alterations in weight resulting from clot disruption. Finally, by measuring the variation in weight before and after the clot lysis, the percentage of clot lysis was calculated and the equation used for this determination:

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

Here, A and B represent the weight of released clot before and after treatment.

2.9 Membrane Stabilizing Activity Test

Preparation of Human Red Blood Cells (HRBC) Suspension: In this research, a sterile Alsever solution consisting of 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water was utilized. The solution was then mixed with an equal volume of freshly drawn human blood. The present study involved the washing of packed cells with iso-saline (0.85%, pH 7.2) for a total of three cycles. Following this, the blood was subjected to centrifugation at a rate of 3000 revolutions per minute for a duration of 10 minutes. In this study, the volume of blood was quantified and subsequently reconstituted as a 10% v/v suspension with an iso-saline solution [17].

Heat induced Hemolysis: The current research centers on the fundamental principle of a particular method, which is based on the stability of the human red blood cell membrane. This stability is achieved through the process of hypotonicity-induced membrane lysis. In this study, a reaction mixture was prepared to consist of 0.15M phosphate buffer [1mL, pH 7.4], 0.36% hyposaline [2mL], 10% v/v HRBC suspension [0.5mL], and plant extracts [0.5mL]. Diclofenac sodium was used as a standard drug, and distilled water was used instead of hyposaline to produce 100% hemolysis, which served as the control group. The reaction mixture was incubated at 37°C for 30 minutes and then centrifuged. The purpose of this experiment was to investigate the effects of the plant extracts on hemolysis and compare them to the effects of diclofenac sodium and the control group. In this study, the hemoglobin content in the suspension was determined using a spectrophotometer at a wavelength of 560nm. The formula used for estimating the percentage of hemolysis of HRBC membrane as follows:

% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100

The equation utilized for determining the percentage of HRBC membrane stabilization:

% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100]

2.10 Acute Toxicity Test

In this experiment, each group consisted of five mice that were administered different doses of EELA and Cinnamon oil orally. The doses provided were 1000 mg/kg, 3000 mg/kg, and 5000 mg/kg. The control group received the vehicle, which was water.

Following a period of 24 hours of continuous monitoring, mortality rates were acquired for each of the experimental groups.

3. RESULTS

3.1 Statical Analysis

The experimental data were subjected to triplicate handling, and the mean and standard deviation were utilized to express the tabular data. The software application known as Excel has been found to be useful in conducting statistical analyses.

3.2 Phytochemical Screening Result

EELA 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	EELA
Alkaloid	++
Carbohydrate	+
Saponin	+
Glycosides	++
Reducing Sugar	+
Flavonoid	++
Tannin	–
Steroid	–
Gum	+
Phenol	++

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

3.3 Anti-inflammatory Activity

The in vitro anti-inflammatory test of plant extracts is a crucial technique for assessing the potential therapeutic advantages of various plants. This method involves the evaluation of the anti-inflammatory properties of plant extracts outside of a living organism. The denaturation test of egg albumin is a commonly employed method to evaluate the anti-inflammatory properties of plant extracts. The results presented in Table 2 demonstrate that the egg extract of Lymantria dispar (EELA) exhibits a greater effect on egg albumin denaturation at concentrations of 1000 and 500 µg/mL when compared to Acetyl salicylic acid, a commonly used anti-inflammatory medication.

3.4 Result of Antiarthritic Activity

Table 3 and Fig. 2 demonstrate a comparison between the denaturation property of Bovine Serum Albumin (BSA) and the standard drug.

3.5 Result of Antifungal Activity

This research investigation aimed to investigate the antifungal activity of various dosages of plant extract against four different types of fungi. The zone of growth inhibition in millimeters was measured as an indicator of antifungal activity. The present study investigated the effect of increasing plant extract concentration on the inhibitory zone. The results obtained from the experiment indicated that as the concentration of the plant extract increased, the inhibitory zone also increased in size. This observation is supported by the data presented in Table 4. The observed zone of inhibition for fungi was found to be within the range of 06-13 mm.

Table 2. Percentage inhibition in egg albumin denaturation of EELA

Samples	Concentrations(µg/mL)	% of inhibition µg/mL
Acetyl salicylic acid	62.5	20
	125	33.33
	250	66.67
	500	77.78
	1000	88.88
EELA	62.5	74.82
	125	81.29
	250	84.17
	500	84.89
	1000	87.05

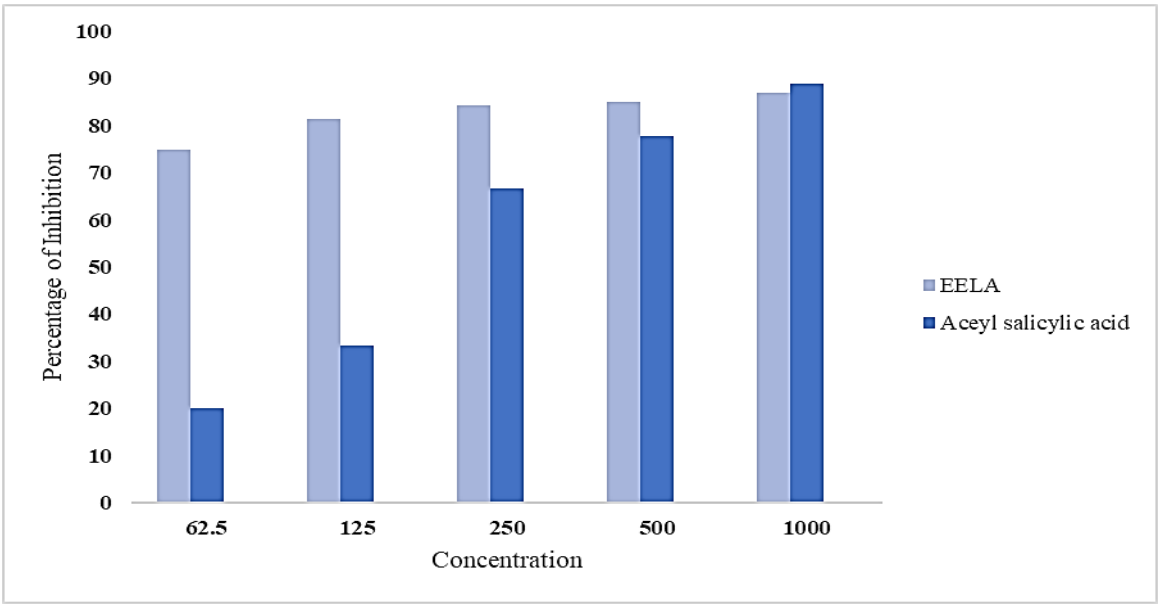

Fig. 1. Percentage of inhibition of EELA compared to standard using egg albumin

Table 3. *In vitro* anti-arthritic test results of EELA

Samples	Concentrations (µg/mL)	% of inhibition
Diclofenac Sodium	62.5	84.64
	125	87.38
	250	89.18
	500	93.69
	1000	94.59
EELA	62.5	80.18
	125	84.68
	250	92.79
	500	94.59
	1000	95.49

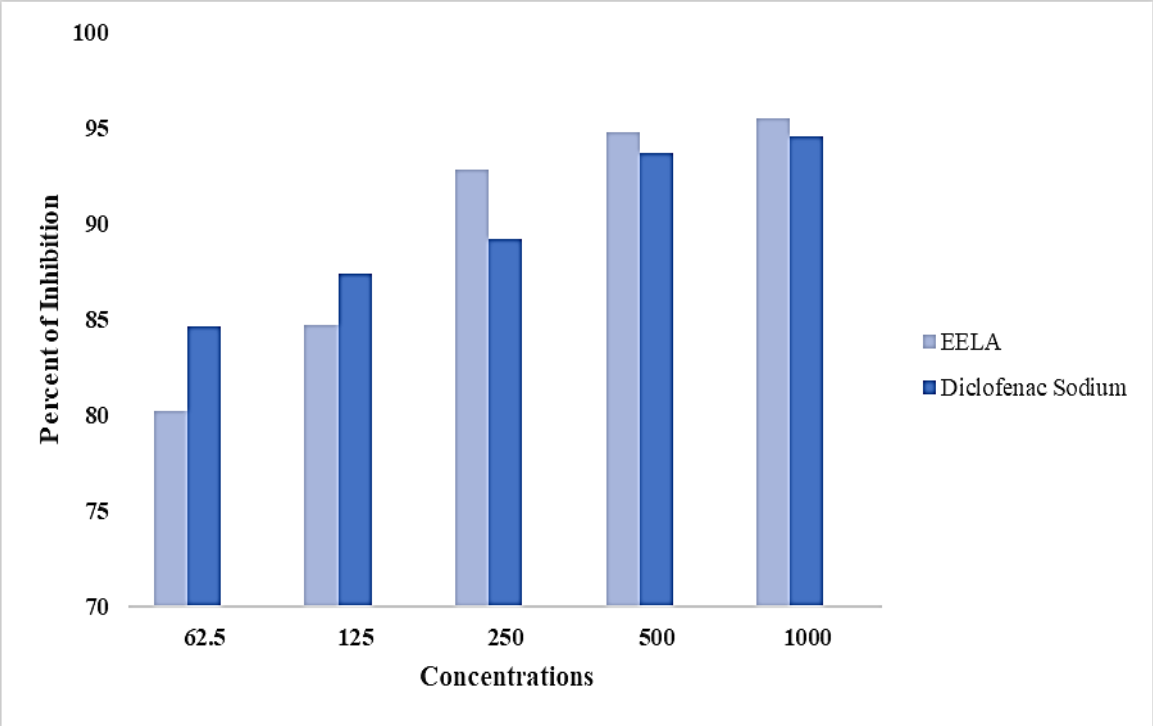

Fig. 2. Percentage of inhibition of EELA compared to standard

Table 4. Results of antifungal activity of EELA (mm)

	Diameter of Zone of Inhibition (mm)			
Test organisms	**EELA (300 µg/disc)**	**EELA (500 µg/disc)**	**EELA (700 µg/disc)**	**Nystatin**
Aspergillus niger	07	09	13	19
Penicillium chrysogenum	07	09	12	22
Mucor hiemalis	06	10	11	22
Yeast budding	07	08	10	20

3.6 Membrane Stabilizing Activity

The current research involves the evaluation of the hemolytic potential of EELA, as compared to the standard. The percentage of hemolysis and the protective effect of EELA are measured using a standardized test, as described in detail in Table 5.

Table 5. Percentage of hemolysis of RBC by EELA extract

Sample	Concentration (µg/mL)	% Hemolysis	% Protection
Standard	500	26.36	73.63
EELA	500	17.97	82.02

Table 6. Percentage of clot lysis, n=10 (mean value)

Sample	% of clot lysis
Negative Control	7.296
Streptokinase	96.301
EELA	91.36

Table 7. Effect of EELA on mice

Sample	Onset time of seizure (s)	Mortality protection after 30min	Mortality protection after 24h
Normal Saline	27±2.95	0/5	0/5
Cinnamon Oil(20mg/kg)	343±3.76	5/5	5/5
EELA (1000mg/kg)	19±2.21	0/5	1/5
EELA (3000mg/kg)	75±3.04	1/5	2/5
EELA (5000mg/kg)	115±2.82	2/5	3/5

3.7 Thrombolytic Activity

The thrombolytic activity of EELA has been found to be significantly greater than that of the standard drug, as evidenced by the data presented in Table 6. The present study suggests that EELA may have potential as a therapeutic agent for reducing blood clots, similar to the drug plasmin.

3.8 Toxicological Activity

In the acute toxicity test, the ethanolic extract was administered at 1000, 3000, and 5000 mg/kg doses, while cinnamon oil was administered at 20 mg/kg. The results showed that the ethanolic extract and cinnamon oil significantly delayed the onset of seizures compared to the negative control group. The mortality protection seen in convulsion survivors varied according to the dosage of ethanolic extract administered. In particular, the rate of convulsion survivors to total animals tested was 4 out of 5 when a dosage of 1000 mg/kg was used, 2 out of 5 when a dosage of 3000 mg/kg was used, and 5 out of 5 when a dosage of 5000 mg/kg was used. These results indicate a significant impact of the ethanolic extract compared to the efficacy of Cinnamon oil, which resulted in a 5 out of 5 convulsion survival mice when administered at a dosage of 20 mg/kg.

4. DISCUSSION

The present investigation was to analyze the impact of EELA on a range of pharmacological assays, including anti-inflammatory, antifungal, anti-arthritic, thrombolytic, and membrane stabilizing activities. Additionally, the phytochemical screening of EELA was conducted to identify its chemical constituents. Table. 1 presents the findings of the study, indicating that EELA exhibits a higher concentration of alkaloids, flavonoids, phenols, and glycosides. Alkaloids are a class of naturally occurring compounds that have been found to possess anti-inflammatory and analgesic properties. These properties have been shown to be effective in reducing pain and enhancing immune response. The *Launaea asplenifolia* plant has been found to contain a notable quantity of alkaloids, which have been identified as having potential medicinal properties for the treatment of skin diseases, asthma, and snake bites. The present study evaluated the nutritional, phytochemical, antioxidant, and cytotoxic potential of EELA. The results indicate that EELA contains a higher amount of saponins, which are

known to exhibit various pharmacological activities such as anti-cancer, antioxidant, antimicrobial, anticonvulsant, anthelmintic, anti-inflammatory, analgesic, and cytotoxic effects. Therefore, the presence of saponins in EELA suggests that this plant can be of medicinal importance. Further research is warranted to explore the potential therapeutic applications of EELA and its active constituents. The analysis of the sample revealed the presence of carbohydrates, reducing sugar, and gum in moderate quantities, as indicated in Table. 1. The presence of flavonoids in various plant-based sources such as fruits and vegetables has been widely documented. These compounds have been shown to exhibit notable antibacterial and antioxidant properties, making them of particular interest in the field of natural product research. According to previous research, the anti-bacterial properties of certain compounds can be attributed to the structures of flavonoids [18]. The identification of phytochemicals in medicinal plants is crucial for understanding their therapeutic potential. In the case of EELA, the presence of a particular phytochemical has been found to be of significant importance. This finding underscores the potential medicinal value of this species. However, it is noteworthy that tannins and steroids were not detected in the screening process. Further research is needed to fully elucidate the chemical composition of EELA and its potential therapeutic applications.

The egg albumin assay aims to evaluate the inhibitory potential of test substances on protein denaturation that is closely linked to inflammation. This research investigated the inhibitory effects of *Launaea asplenifolia* extracts on protein denaturation using the egg albumin assay. The results obtained from the experiment demonstrated that EELA exhibited significant inhibitory effect 87.05% on protein denaturation at the concentration of 1000 µg/mL which is nearly equal value of the standard drug Diclofenac sodium's value 88.88%. The present study reports on the inhibitory effects observed in relation to the ability of the extracts to safeguard the protein structure and prevent its precipitation. The findings indicate that the plant may exert its anti-inflammatory effects by interfering with protein denaturation processes that are known to be involved in the inflammatory response. This property can be attributed to the presence of bioactive compounds such as flavonoids, phenolics, and terpenoids. These compounds have been reported to exhibit anti-inflammatory properties, which may contribute to the plant's therapeutic potential [16]. Specifically, the compounds have demonstrated the ability to inhibit the production of pro-inflammatory mediators such as cytokines (IL-6, TNF-α), enzymes (COX, LOX), and NO. In addition to their other functions, it has been observed that they possess the ability to modulate the activation of NF-κB, a transcription factor that plays a crucial role in the inflammatory response [20].

The anti-inflammatory activity of related species within the *Launaea* genus has been reported in previous studies. The present study aims to investigate the anti-inflammatory potential of *Launaea mucronata* by analyzing its inhibitory effects on carrageenan-induced paw edema in rats. The aforementioned statement provides evidence to suggest that there is a possibility of other species belonging to the *Launaea* genus possessing anti-inflammatory properties [20].

The antifungal activity of EELA was evaluated against four different fungi, as presented in Table 4. As per the findings presented in Table 4, it can be observed that EELA demonstrates varying degrees of antifungal efficacy against different types of fungi. The present study reports on the comparative fungicidal activity of EELA and griseofulvin against *yeast*-like fungi. The results indicate that EELA exhibits stronger fungicidal activity than griseofulvin. The present study investigated the antimicrobial activity of *Penicillium chrysogenum*, *Aspergillus niger*, and *Mucor hiemalis*. The zone of inhibition was measured and compared to the standard. Results indicate that the zone of inhibition for all three fungi was found to be in close proximity to the standard [21]. It can be concluded that EELA has the potential to function as an antifungal agent.

Rheumatoid arthritis (RA) is a type of inflammatory disease that affects approximately 1% of the population in economically developed countries. Acute rheumatoid arthritis (RA) is characterized by a triad of symptoms, namely, limited mobility, hyperalgesia, and cessation of body weight gain [22]. The present study demonstrates the anti-arthritic potential of EELA, as evidenced by a significant value of 95.49% at a concentration of 1000µg/mL. This value is comparable to that of Diclofenac sodium, which exhibited a value of 98.19% at the same concentration (Table 3, Fig. 2). The potential therapeutic application of the substance in question, due to its noteworthy anti-arthritic

properties, may warrant further investigation for the treatment of Rheumatoid arthritis in forthcoming medical research.

In this research, the membrane stabilization percentages of EELA and Diclofenac sodium were determined through the inhibition of HRBC membrane lysis. The results presented in Table 5 demonstrate the efficacy of EELA in suppressing the hemolysis of HRBC induced by heat. The results indicated that *Launaea asplenifolia* exhibited a higher range of protection (82.02%) compared to Diclofenac sodium (73.63%). These findings suggest that *Launaea asplenifolia* possesses a significant membrane stabilizing property. The present study posits that the observed activity can be attributed to flavonoids. The potential anti-inflammatory properties of *Launaea asplenifolia* have been identified and studied. It is suggested that this plant species may have the ability to act as an effective anti-inflammatory agent [23].

This study examined thrombolytic drugs' ability to dissolve clots. To do this, a thrombolytic test contrasted a positive control to a negative control. As shown by the test, adding water to the clot did not dissolve it. Compared to streptokinase (SK) and water, EELA's clot lysis % was much higher. This research shows that plants have bacterial contamination with plasminogen receptors. The cell surface helps activate plasminogen to plasmin, which may cause fibrinolysis [24]. The bacterial plasminogen activator streptokinase helps generate exosites as a cofactor molecule. Substrates are better presented to the enzyme. Bacterial plasminogen activators' involvement in enzymatic pathways is affected by these results. Clots dissolve when Staphylokinase activates plasminogen. Staphylokinase may also destroy cell adhesion-causing extracellular matrix and fibrin fibers. Various research, including [25], [26], have found these results. This research reveals a link between thrombolytic effects and the antibacterial activity of the investigated plants. Based on the above, investigating EELA's clot-lytic process would be quite interesting.

From the phytochemical screening, it was found that EELA contained significant number of alkaloids. Hence, the toxicological properties of EELA may be influenced by the chemical composition and levels of aspidosperma-type alkaloids [27].

5. CONCLUSION

The ethanolic extract of *Launaea asplenifolia* has been investigated for possible pharmacological effects. The extract was tested for anti-inflammatory, antifungal, anti-arthritic, membrane stabilizing, and thrombolytic properties in-vitro. The ethanolic extract of *Launaea asplenifolia* showed in vitro anti-inflammatory, antifungal, anti-arthritic, membrane stabilizing, thrombolytic and in-vivo toxicological properties. These results support *Launaea asplenifolia* as a natural source of pharmacologically active chemicals. To understand the processes and assess in vivo effectiveness and safety, further research is needed. The phytochemical studies on the extract found decreasing sugar, saponins, tannins, flavonoids, alkaloids, and glycosides. These bioactive compounds may use as API of new drugs. These chemicals' pharmacological properties and possible use in medication development require further study. This research examined EELA's antifungal properties. EELA showed trace antifungal action. EELA's antifungal potential needs more study. The Protein denaturation assay was tested for anti-inflammatory and anti-arthritis effects. Research showed a considerable impact. Compared to streptokinase, the extract is thrombolytic. Membrane stabilization also has important features. From its acute toxicological activity test its proven that this plant has toxicological effect on animals in higher doses. Researchers may utilize GC-MS analysis, column chromatography, NMR, and in-vivo tests to confirm results.

ETHICAL APPROVAL AND CONSENT

The present study adhered to the guidelines established by the US Food and Drug Administration, the Declaration of Helsinki, and the International Conference on Harmonisation. The present study was conducted under the auspices of the Faculty of Science at Stamford University Bangladesh. The research protocol and informed consent form were thoroughly scrutinised and approved by the institutional ethics review committee (reference number: SUB/ERC/202302). In accordance with ethical research practises, all participants in the study were required to provide a documented consent form prior to their involvement. Additionally, participants were informed of their right to withdraw from the study at any point in time.

ACKNOWLEDGEMENT

The authors express their gratitude to the Dhaka Lab, Bangladesh Council of Scientific and Industrial Research, Laboratory of Microbiology and the Laboratory of Physiology and

Pharmacology the Faculty of Science, Stamford University Bangladesh for their valuable assistance, provision of resources, and infrastructural facilities that were essential for conducting this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://doi.org/10.1007/s13659-020-00252-2

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/109897