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Anti-Arthritic, Anti-Inflammatory, Thrombolytic, Membrane Stabilizing, Antifungal, Cytotoxic And In Vivo Acute Toxicological Activity Of Pandanus Tectorius Leaf Extract

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ABSTRACT
The primary aim of this research was to examine the distinct in-vitro and in-vivo characteristics of plant extracts obtained from Pandanus tectorius via the use of the methanol extraction technique. The main objective of this study was to evaluate the methanolic extract of Pandanus tectorius (MEPTL) using in vitro assays to determine its potential for various activities such as anti-arthritic, anti-inflammatory, thrombolytic, membrane stabilising, antimicrobial cytotoxic activity, and antinociceptive activities. The situation under consideration may be ascribed to the pharmaceutical industry's notable inclination towards the diverse constituents of the plant. The study used the Bovine Serum Albumin Protein Denaturation Assay to evaluate the anti-arthritis efficacy at different doses. The Egg Albumin Protein Denaturation Assay was used to quantify the anti-inflammatory efficacy. The researchers used the disc diffusion technique to evaluate the antibacterial efficacy of the plant being studied. The clot lysis test was used to quantify the thrombolytic activity. The researchers used the heat-induced hemolysis test as a means to evaluate the activity of membrane stabilisation. Furthermore, the use of the Brine Shrimp Lethality test was performed as a means to ascertain the cytotoxic properties of the plant in question. The measurement of acute toxicity was conducted using the Cinnamon oil-induced technique. The study results suggest that MEPTL has significant arthritic action, as shown by a 94.69% inhibition rate at a concentration of 1000 µg/mL. An 87.33% suppression of inflammation was seen when the same dosage was administered. The study revealed a thrombolytic activity of 77.32%, whereas the membrane stabilising activity was determined to be 57.82%. Furthermore, the antimicrobial test demonstrated a dose-dependent zone of inhibition that varied between 07-19 µg/disc. The cytotoxic experiment yielded a very strong LC50 value of 1.057. The results of the acute toxicity test revealed a significant increase in mortality among the mice after administration of a dosage of 3000mg/kg. In summary, the phytochemical present in this plant has strong pharmacological properties, making it a potentially valuable candidate for further investigation in several fields of drug research.

Keywords: *Anti-arthritic; thrombolytic; membrane stabilizing; antifungal; cytotoxic activity.*

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I. INTRODUCTION

A large number of today's modern medicines are derived from the plant resources. In the past, people uses different plant parts for therapeutic purposes (Vickers and Zollman, 1999). Herbal drugs are used because there is widespread faith and reliance on this type of drugs for being cheap and convenient; also, do not contain any adverse effect. Besides, many of the few potent medicines were plant derived. Examples include morphine (originated from the opium poppy), digoxin (originated from foxglove), aspirin (originated from willow bark), and quinine (originated from cinchona bark) (Pal and Shukla, 2003). Since pharmacology enlarged itself such a guiding principle of curative therapy, the use of herbal treatment went into prompt decline. But this scenario has begun to change over the span of years. Nowadays herbal medicines are extensively used by widespread US population and it is expanded by 380% between 1990 and 1997 (from a 1-year frequency of 2.5–12.1%), as an example (Ernst, 2005). A number of chronic or persistent diseases like arthritis, diabetes, AIDS or cancer are often not possible to cure with allopathic system of medicine and for this reason, people are now depending on herbal medicine (Gaidhani, Harwalkar and Nirgude, 2014). On the contrary, herbal medicines can also have some interactions and for this reasons doctor, pharmacists and many other health care professionals should be well informed to consult laibly to the patients (Tuomilehto, no date).

Polyscias scutellaria Locally referred to as "Pandan Laut," this mangrove coastal plant from the Pandanaceae family, which has about 700 species and is found worldwide in subtropical and tropical regions including Malaysia and Indonesia, it is also known by its botanical names of *P. odoratissimus*, *P. fascicularis*, and *P. amaryllifolius*. The distinctive composite edible fruits are composed of discrete components called "keys" joined to a fibrous core. "Bunch" refers to the complete composite fruit, including the core and keys. For their luscious pulp, the interiors of the keys are bitten and sucked. Indian Ayurvedic medicine has historically prescribed *P. tectorius*, particularly its leaves, for treating numerous ailments. The chemical constituents of natural substances directly affects their medicinal effects. Traditional uses for pandanus oils include treating earaches, headaches, arthritis, debility, giddiness, rheumatism, smallpox, and spams. The essential oils found in its leaves include ether (37.7%), terpene-4-ol (18.6%), a-terpineol (8.3%), 2-phenylethyl alcohol (7.5%), benzyl benzoate (11%), viridine (8.8%), and germacrene (8.3%), as well as tiny amounts of benzyl salicylate, benzyl acetate, and benzyl alcohol. Additionally, *P. tectorius* leaves are used to cure cancer, boils, hepatitis, dysuria, and boils. Alkaloids, flavonoids, saponins, and phenols (vanillin, benzoic acid methyl ester, and a novel benzofuran derivative) were abundant in it. Physcion, daucosterol, b-sitosterol, camphosterol, palmitic acid, and steric acid in the rhizomes, while terpenes (phytosteroid mixture), eudesmin, kobusin, desmin, and hydrofuran are present in leaves of this plant. Its root preparations included high concentrations of lignans, benzofuran derivative, a-terpineol, b-carotene, b-sitosterol, vitamin C, tangerine, germacrene-B, and vanidine, all of which are potent antioxidants. The antioxidant compounds found in the *P. tectorius* fruit extract, such as caffeoylquinic acids, vitamins C, E, and b-carotene, isopentenyl, dimethylallyl acetates, and cinnamates, might all be linked to the biological effectiveness of the extract. The pharmacological effects of *P. tectorius* include hypolipidemia, cholesterol-lowering action, anti-inflammatory, antioxidant, anti-cancer, anti-tumor, and antiviral properties. Additionally, the caffeoylquinic acid-rich *P. tectorius* fruit extract's antihyperglycemic and antihyperlipidemic activities were looked at. They demonstrated that in diabetic db/db mice, this extract improves insulin sensitivity and controls hepatic glucose and lipid metabolism.

The motive behind this analysis is to find out in vitro anti-arthritic, anti-inflammatory, thrombolytic, membrane stabilizing, antifungal and cytotoxic activities of *P. tectorius* from its methanolic leaf extract along with its phytochemical screening.

II. MATERIALS AND METHODS

Plant Materials

The sample plant *P. tectorius* was collected in March 2023 from St. Martin Iceland, Bangladesh. Then the plant (accession number: DACB 88046) was precisely recognized by the professionals at the Bangladesh National Herbarium in Mirpur, Dhaka. In the meantime, plant's leaves had been stored and dried in shade and powder was made from these dried leaves.

Reagents

Methanol, concentrated H_2SO_4, Diluted HCl acid, acetic acid and NaOH was supplied by Sigma Chemical Co., USA. From Polysciences, Inc. India, Bovine Serum Albumin was bought. Streptokinase was purchased from Incepta Pharmaceuticals Ltd, Bangladesh. Square Pharmaceuticals Ltd manufactures diclofenac sodium injections. The sterile saline solution was obtained through Orion Infusion Ltd. Vincristine Sulphate was taken from Celon Laboratories Pvt. Ltd. India.

Preparation of Plant Extract

The extraction of plant was obtained by using cold maceration method (Nn, 2015). About 90g powder of *P. tectorius* leaf was soaked in 700 mL of methanol for 12 days in a round bottom flask sealed with a stopper and wrap (Wu *et al.*, 2015). Then the mixture was filtered and air dried for further 8 days. After drying, overall weight of 17.23g of leaf extract was obtained.

In Vitro Anti-Arthritic Test

Rheumatoid arthritis (RA) is one of the prevalent autoimmune disorder which is accompanying with systemic difficulty, progressive impairment, premature death and socioeconomic cost (Alivernini, Firestein and McInnes, 2022). About 0.3-1% people across the world are effected by rheumatoid arthritis and among them males are three times less prone to RA then females (Choudhary *et al.*, 2015).

Anti-arthritic activity is tested by using protein denaturation assay by bovine serum albumin method (P, M and B, 2019). Bovine serum albumin (5% aqueous solution) of 0.45 mL and MEPSL of 0.05 mL are together formed 0.5 mL of test solution and as a standard drug, 0.05 mL of Diclofenac sodium were used. MEPTL and Diclofenac sodium are sampled in different concentration (62.5, 125, 250, 500, 1000 µg/mL). Small amount of 1N HCl is added to modify the pH of the solution to 6.3. After that, for 20 minute at 37^{o}C the samples were incubated and heated for 3 minute at 57^{o}C. Then 2.5 mL phosphate buffer was added after cooling the solution.

Finally, at a wavelength of 416 nm the absorbance of the solutions was taken by using UV-Visible spectrometer. Here, 0.05 mL of distilled water is used as a test control instead of utilizing BSA (Bovine Serum Albumin) for control. For comparison, Diclofenac sodium is used in this study. Equation used for calculating percentage of inhibition of protein denaturation:

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

Here, OD means optical density.

In Vitro Anti-Inflammatory Test

For this test, various concentrations of 62.5, 125, 250, 500 and 1000 µg/mL mixture was prepared which consist of total 5 mL of reaction mixture containing 2.8 mL of phosphate buffered saline (PBS, pH 6.4), 0.2 mL of egg albumin (from a hen's egg), and 2 mL of MEPTL. Double-distilled water was used at equal amount for control group. At 70ºC, the mixtures were heated for 5 minutes after incubating the mixture at (37±2)ºC, using Biological Oxygen Demand (BOD) incubator for a time period of 15 minutes. After cooling, absorbance of the mixtures was taken at 660 nm. For comparison, Acetyl Salicylic acid also used in equal concentration as a standard (Alamgeer, Uttra and Hasan, 2017). Fractional equation for calculating percentage of inhibition of protein denaturation:

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

In Vitro ThrombolyticTest

Blood Sample:

4 mL of venous blood was drawn from healthy human volunteers (n=15), whom had never consumed blood thinners, nicotine and oral contraceptives, and this process was aided by a medical professional. The whole process was received ethical approval from the Institutional Ethics Council of Stamford University Bangladesh. Then, total of 15 micro centrifuge tubes was filled with 500 µL of fresh blood.

Affirmation of Donors consent:

Every single donor was supplied with a consent form that narrated the purpose of this research, title of this project, and the volume of blood that will be drawn. The illustration of this research includes whether or not volunteers will consume any therapy, any kind of irritation to the piercing area and the time period for blood collection.

Clotlysis method:

The method used for determining the percentage of clotlysis was obtained previously published research paper (Umesh *et al.*, 2014). In short, 2.5 mL of fresh blood was filled in 15 discrete pre-weighed sterile micro centrifuge tubes (0.5 mL/tube) and at 37ºC, it was incubated for 45 minutes. After incubation, serum was deliberately drained out from the tubes without disturbing the clot. For calculating the clot weight, tubes were weighted again (Clot weight= weight of clot containing tube – weight of tube without clot). Then 100 µL of MEPTL was added to each micro centrifuge tube which contain pre-weighted clot. By adding 2.5 mL of PBS, lyophilized streptokinase vial was reconstituted and was mixed properly. In the volume of 100 µL of this suspension was filled to the tube as a positive control. For negative control, distilled water of 100 µL was used. Clotlysis was checked in each tube after incubating at 37ºC for 90 minutes. After incubation, the tubes were weighted again to observe the weight changed for clot disruption. Finally, by measuring the variation in weight before and after the clotlysis, the percentage of clotlysis was calculated and the equation used for this determination:

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

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

Membrane Stabilizing Activity Test

Preparation of Human Red Blood Cells (HRBC) Suspension:

2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water used for making a sterile Alsever solution which was mixed with equal quantity of freshly sampled human blood. Then centrifugation of that blood was performed at 3000 rpm for 10minutes and combining with isosaline (0.85%, pH 7.2), packed cells were washed three times. Reconstitution as 10% suspension was performed with isosaline and the volume of blood was measured (Chippada *et al.*, 2011).

Heat induced Hemolysis:

The fundamental principle of this method is the stability of human red blood cell membrane through hypotonicity induced membrane lyis. As reaction mixture, 0.15M phosphate buffer [1 mL, pH 7.4], 0.36%

hyposaline [2 mL], 10% v/v HRBC suspension [0.5 mL] with plant extracts [0.5 mL] and diclofenac sodium used as a standard drug and distilled water instead of hypo saline to produce 100 % hemolysis used as a control group and incubation performed at 37°C for 30 min and centrifugation respectively. Spectrophotometer at 560 nm was used to determine the hemoglobin content in the suspension. 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]

Antimicrobial Susceptibility Test

Fungal strains:

From Microbiology Department of Stamford University Bangladesh, pure culture of fungi (*Penicillium chrysogenum*, *Aspergillus niger*, *Mucor hiemalis* and *Saccharomyces cerevisiae*) was obtained.

Disc Diffusion Method:

Antimicrobial activity of MEPTL was carried out by using disc diffusion assay (Klančnik *et al.*, 2010). In this method, a solid agar medium was formed in a Petri Dish. Then 1 mL culture of each fungus was spread uniformly throughout the medium. Sterile filter paper disc of 6 mm in diameter was used and this disc was saturated with diluted MEPTL of 10 µL, setting on the top of each agar plate. In this test, MEPTL was taken in several concentration (300, 500, 700 µg/mL). Then the plates were put on the incubator for next 24 hours. Griseofulvin containing disc was used as an antifungal agent for positive control, while methanol containing disc was used for negative control. After 24 hours, based on the size of inhibition zone surrounding the disc, measured in mm, antifungal activity was determined (Singh, Zaman and Gupta, 2007).

In Vitro Cytotoxic Activity Test

Cytotoxic activity of MEPTL was investigated using the brine shrimp lethality test, a standard bioassay for screening bioactive compound ('Evaluation of nutritional, phytochemical, antioxidant and cytotoxic potential of', 2021). *Artemia salina* (zoological organism) used as a model for this research. At first, from a pet store (Dhaka, Bangladesh) shrimp eggs were bought. Hatching of shrimp eggs were performed in artificial seawater (3.8% NaCl solution) after incubating 48 hours in it and larval shrimp (nauplii) was grown. By applying Meyer's approach brine shrimp nauplii can be evaluated for cytotoxic activity. Test sample of MEPTL was prepared by dissolving it in a dimethyl sulfoxide solution that cannot be more than 50 µL per 5 mL. Then artificial seawater was mixed up to 5 mL for making desirable concentration (1.95, 3.91, 7.81, 15.625, 31.25, 62.5, 125, 250, and 500 µg/mL). For positive control, Vincristine sulphate was employed. Then 10 mature shrimp nauplii were added in test tube. Test tubes were observed by using magnifying glass after 24 hours to see how many nauplii had survived. By utilizing a logarithmic plot of concentration against mortality rate, LC_{50} was calculated.

Acute toxicity test

5 mice in each group were given either 1000 mg/kg, 2000 mg/kg, or 3000 mg/kg of MEPTL and Cinnamon oil orally, with the vehicle (water) serving as a control. After 24 hours of observation, death rates were obtained for both groups (Franzotti et al., 2000).

Statistical Analysis

All experimental data were handled in triplicate, and mean, standard deviation was used to express tubular data. Excel also used for statistical analyses.

III. RESULTS

In Vitro Anti-Arthritic Test:

Denaturation of BSA property compared to the standard drug has been shown in Table 1.

Table 1. Invitro anti-arthritic test results.

Samples	Concentrations (µg/mL)	% of inhibition
Diclofenac Sodium	62.5	89.19
	125	91.89
	250	93.69
	500	94.59
	1000	98.19
MEPTL	62.5	82.77
	125	83.69
	250	86.37

	500	92.49
	1000	94.69

In Vitro Anti-Inflammatory Test:

The percentage of proteinase inhibition carried out by MEPTL shows a dose dependent rise which is in moderate level compared to the standard and shown in Table 2.

Table 2. Protein denaturation (egg albumin) assay results

Samples	Concentrations (µg/mL)	% of inhibition
Acetyl Salicylic acid	62.5	93.52
	125	94.96
	250	95.68
	500	97.84
	1000	98.56
MEPTL	62.5	79.88
	125	81.56
	250	82.09
	500	83.74
	1000	87.33

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In Vitro ThrombolyticTest:

Thrombolytic activity of MEPTL is very significant than compared to standard drug (Table 3). So, it can be assumed that MEPTL can be used as a drug like plasmin which can reduce blood clots.

Table 3. Percentage of clot lysis, n=10 (mean value)

Sample	% of clot lysis
Negative control	7.296
Streptokinase	91.304
MEPTL	77.32

Membrane Stabilizing Activity Test:

Percentage of hemolysis and protection of MEPTL compared to the standard is measured in this test which is deliberated in Table 4.

Table 4. Percentage of hemolysis of RBC by MEPTL extract

Sample	% of hemolysis	% of protection
Diclofenac Sodium	26.36	73.63
MEPTL	42.18	57.82

Antifungal Susceptibility Test:

A moderate antifungal activity has obtained compared to the standard drug which is demonstrated in Table 5.

Table 5. Results of antifungal activity of MEPSL (mm)

Diameter of Zone of Inhibition (mm)				
Test organisms	**MEPSL (300 µg/disc)**	**MEPSL (500 µg/disc)**	**MEPSL (700 µg/disc)**	**Griseofulvin (50µg/disc)**
Penicillium chrysogenum	**07**	**08**	**10**	**19**
Aspergillus niger	**07**	**11**	**13**	**20**
Mucor hiemalis	**08**	**10**	**15**	**21**
Saccharomyces cerevisiae	**10**	**20**	**36**	**21**

In Vitro Cytotoxic Activity Test:

In **Table 6.** The cytoxic activity of MEPSL to brine shrimp nauplii is summarized and standard calibration curve of standard and MEPTL, which shows the effect of both on brine shrimp nauplii, illustrated in **Figure 1** and **Figure 2** respectively.

Table 6. Brine Shrimp Assay (Mortality %, LC_{50} value)

Sample	Concentration (C) (µg/mL)	Mortality %	LC_{50} value
Vincristine Sulphate	1.95	40	0.608
	3.91	40	
	7.81	60	
	15.325	70	
	31.25	80	
	62.5	90	
	125	90	
	250	100	
	500	100	
MEPTL	1.95	20	1.057
	3.91	30	
	7.81	40	
	15.625	60	
	31.25	70	
	62.5	80	
	125	90	
	250	100	
	500	100	

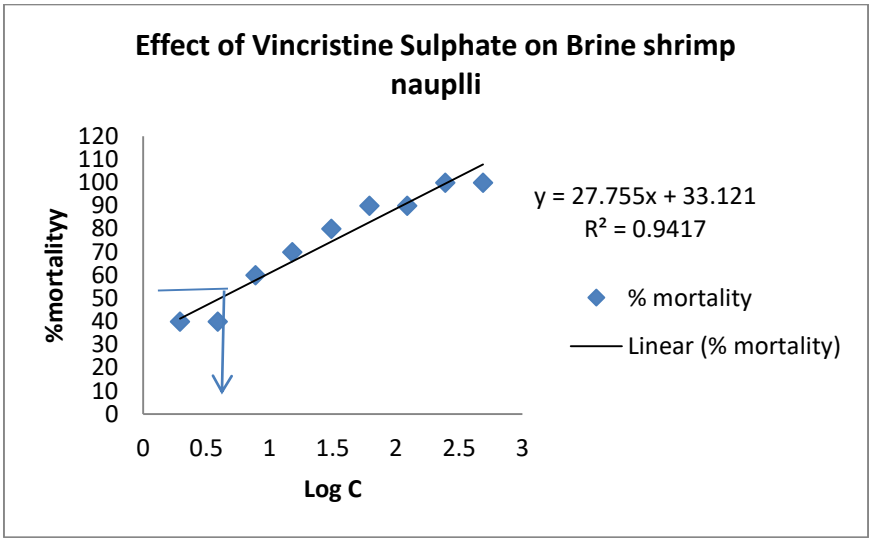

Fig 1. Cytotoxic activity of Vincristine Sulphate on Brine shrimp nauplii

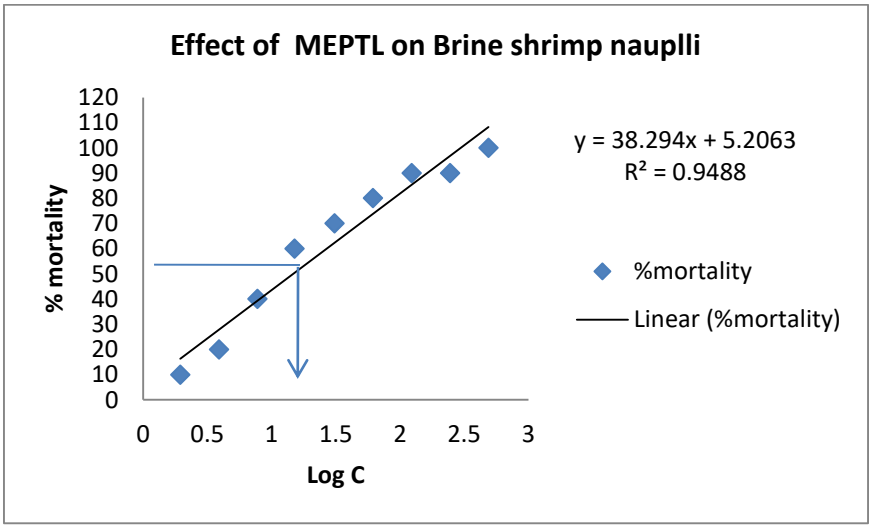

Fig 2. Cytotoxic activity of MEPTL on Brine shrimp nauplii

Acute Toxicity

In the acute toxicity test, administration of the methanolic extract at dosages of 1000, 2000, and 3000 mg/kg as well as cinnamon oil at a dose of 20 mg/kg prolonged the start time of seizure in comparison to the negative control group. The rate of convulsion survivors to total animals tested (mortality protection) was 4/5 in 1000 mg/kg methanolic extract; 3/5 in 2000 mg/kg methanolic extract; and 3/5 in 3000mg/kg methanolic extract, which was less effective than the effectiveness of Cinnamon oil (5/5 in 20 mg/kg).

Table 7. Effect of MERP on mice.

Treatment	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
MEPTL (1000mg/kg)	19±2.21	0/5	1/5
MEPTL (3000mg/kg)	75±3.04	2/5	2/5
MEPTL (5000mg/kg)	115±2.82	3/5	3/5

IV. DISCUSSIONS

This investigation has performed for determining the effect of MEPSL on variety of pharmacological tests such as anti-arthritic, anti-inflammatory, antifungal, membrane stabilizing, thrombolytic and cytotoxic activity along with its phytochemical screening. In **Table 1**, it has been shown that alkaloids, carbohydrates, saponin are present at a higher amount on MEPSL. Alkaloids posses' anti-inflammatory and analgesic properties which helps in reducing pain and enhances immune response. MEPSL has significant quantity of alkaloids which can be used to skin diseases, asthma and snake bite. The appearance of saponins in higher amount on MEPSL is an excellent indication that this plant can be used as a medicinal importance because saponin shows anti cancer, antioxidant, antimicrobial, anticonvulsant, anthelmintic, anti-inflammatory, analgesic, and cytotoxic effect ('Evaluation of nutritional, phytochemical, antioxidant and cytotoxic potential of', 2021). Glycosides, reducing sugar, and flavonoids are also present in a moderate amount (**Table 1**). Flavonoids are generally found in plants, fruits and vegetables which posses antibacterial and antioxidant properties. Flavonoids structures are responsible for anti-bacterial characteristics (Zannah *et al.*, 2017). The existence of this phytochemical in MEPSL exerts the medicinal importance of this species whereas; tannins and steroids are not identified in this screening process.

In economically evolved countries, about 1% of populations are affected by Rheumatoid arthritis (RA) which is one type of inflammatory disease. Lack of mobility, hyperalgesia, and pause in body weight gain are the signs of acute RA (Amresh, Singh and Rao, 2007). In this investigation, it has been shown that the MEPSL has significant anti-arthritic value of 94.59% in the concentration of 1000 µg/mL, which is very close when compared to Diclofenac sodium's value of 98.19% in 1000 µg/mL concentration (**Table 2, Figure 1**). Because of its significant anti-arthritic value, it can be used to treat Rheumatoid arthritis in future.

Inflammation is physiologic response to tissue injury and infection; it occurs due to the production of prostaglandins through cyclooxygenase pathway. In this in vitro anti-inflammatory test, we found that the MEPSL has the properties of inhibiting inflammation 86.33% in the doses of 1000 µg/mL, by a percentage close to the inhibition emerged by the extensively recognized NSAIDS such as aspirin (acetyl salicylic acid) 98.52% in the doses of 1000 µg/mL (**Table 3, Figure 2**).Aspirin is the oldest class of NSAID which targets and inhibit cyclooxygenase (COX) pathway, the rate limiting enzyme in the production of prostaglandins. As the MEPSL has inhibition value close to the Acetyl salicylic acid, this study clearly manifested that the MEPSL has cyclooxygenase inhibitory properties by inhibiting in vitro conversion of arachidonic acid to PGE2 (Vázquez *et al.*, 1996).

Different kinds of research have been carried out to determine which supplements, herbs and natural food sources have thrombolytic activity to treat coronary events and strokes. This investigation determined the thrombolytic potential of MEPSL. Thrombolytic potential of MEPSL was rapid and the value is 97.32% compared to standard 91.304% (**Table 4**). This value obtained because MEPSL diminish coagulation of human blood in vitro, so it can be claimed as cardio protective. As the MEPSL has significant value, it may have important implication in cardiovascular health and this may lead to the formation of novel thrombolytic agents from *Polyscias scutellaria* leaf (Ratnasooriya, Fernando and Madubashini, 2008).

The percentage of membrane stabilization for MEPSL and Diclofenac sodium were done by the inhibition of HRBC membrane lysis i.e., stabilization HRBC membrane induced by hypotonicity. MEPSL are efficacious in suppressing the heat induced hemolysis of HRBC as shown in **Table 5**. This indicated the range of protection 58.87% of MEPSL compared to Diclofenac sodium 73.63%, which declare the considerable

membrane stabilizing property of *Polyscias scutellaria* leaf. It can be said that flavonoids are responsible for this type activity. Hence, *Polyscias scutellaria* can be used as an anti-inflammatory agent.

Antifungal activity of MEPSL was shown in **Table 6**, using 4 fungi. According to Table 6, MEPSL exerts several degrees of antifungal activity for each fungus. It was found that MEPSL have stronger fungicidal activity than Griseofulvin against *Saccharomyces cerevisiae* like fungi. In case of *Penicillium chrysogenum*, *Aspergillus niger*, *Mucor hiemalis*, it was found that the zone of inhibition is close to the standard. So it can be said that MEPSL can be used as an antifungal agents (Sasaki, Abe and Yoshizaki, 2002).

Brine shrimp assay is low cost and simple method for determining cytotoxic properties of plant extract. The cytotoxic activity of MEPSL was tested by this method and the results are summarized in **Table 7**. The LC_{50} values for MEPSL, and standard drug Vincristine Sulphate was 1.057 µg/mL and 0.608 µg/mL respectively (**Figure 3 and Figure 4**). Moreover, several dosage levels of test solution were shown to have several degrees of mortality to *Artemia salina*. The values of LC_{50} ranged from 1.95 µg/mL (significant) to 500 µg/mL (very significant), declaring a genuine connection between concentration and LC_{50}. Percentage mortality was highest at a concentration of 500 µg/mL and conversely lowest at a concentration of 1.95 µg/mL. So it can be said, when the concentration of the test samples rises, the percentage of mortality also increases and vice versa. Compared to the standard vincristine sulphate (0.608 µg/mL), the MEPSL exhibit substantial cytotoxic activity against brine shrimp nauplii with LC_{50} value of 1.057 µg/mL. The MEPSL shows significant cytotoxicity compared to the standard vincristine sulphate which can be taken into consideration for further research to be used as an antitumor and pesticides compound (Suffredini *et al.*, 2006).

The results of this research suggest that the MEPTL exhibit significant antinociceptive activity in both central and peripheral regions. The aforementioned extracts exhibited efficacy in mitigating acute toxicity. Regarding the incidence of toxicity, it was observed that MEPTL exhibited a higher degree of toxicity at elevated dosages. Based on a toxicity classification, it can be inferred that the aforementioned extracts possess a relatively high level of toxicity (Hossein et al., 2002).

V. CONCLUSION

As in many other countries, *Polyscias scutellaria* can be found growing wild in Bangladesh. The above description makes it very evident that *Polyscias scutellaria* is rich in phytochemicals and serves several pharmacological purposes. Previously, it was hypothesized that the crude methanolic extract of *Polyscias scutellaria* would have anti-inflammatory, anti-arthritic, and cytotoxic activities; the current research confirms that these hypotheses are correct. Compared to the standard Griseofulvin, the extract showed significant fungicidal activity against some yeast like fungi. Thrombolytic properties of the extract are also remarkable than the standard streptokinase. Membrane stabilizing properties are also significant. This data suggests that *Polyscias scutellaria* may have potential in the pharmaceutical industry. This makes the plant an excellent candidate for more systematic, chemical, and biological testing to isolate the active ingredient. It is possible that GC-MS analysis and in-vivo studies may be required in the future for confirmation by researchers.

VI. ACKNOWLEDGEMENT

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VII. CONFLICTS OF INTEREST

This research didn’t receive any external funding.

VIII. 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/202302). Everyone who took part in the study had to submit a documented consent form, and they had the right to withdraw at any moment.

REFERENCE:

[1]. Alamgeer, Uttra, A.M. And Hasan, U.H. (2017) ‘Anti-Arthritic Activity Of Aqueous-Methanolic Extract And Various Fractions Of Berberis Orthobotrys Bien Ex Aitch’, BMC Complementary And Alternative Medicine, 17(1), Pp. 1–16. Available At: Https://Doi.Org/10.1186/S12906-017-1879-9.

[2]. Alivernini, S., Firestein, G.S. And Mcinnes, I.B. (2022) ‘The Pathogenesis Of Rheumatoid Arthritis’, Immunity, 55(12), Pp. 2255–2270. Available At: Https://Doi.Org/10.1016/J.Immuni.2022.11.009.

[3]. Amresh, G., Singh, P.N. And Rao, C. V. (2007) ‘Antinociceptive And Antiarthritic Activity Of Cissampelos Pareira Roots’, Journal Of Ethnopharmacology, 111(3), Pp. 531–536. Available At: Https://Doi.Org/10.1016/J.Jep.2006.12.026.

[4]. Chippada, S.C. Et Al. (2011) 'In Vitro Anti Inflammatory Activity Of Methanolic Extract Of Centella Asiatica By HRBC Membrane Stabilisation', Rasayan Journal Of Chemistry, 4(2), Pp. 457–460.

[5]. Choudhary, M. Et Al. (2015) 'Medicinal Plants With Potential Anti-Arthritic Activity':, Journal Of Intercultural Ethnopharmacology, 4(2), P. 147. Available At: Https://Doi.Org/10.5455/Jice.20150313021918.

[6]. Ernst, E. (2005) 'The Efficacy Of Herbal Medicine - An Overview', Fundamental And Clinical Pharmacology, 19(4), Pp. 405–409. Available At: Https://Doi.Org/10.1111/J.1472-8206.2005.00335.X.

[7]. 'Evaluation Of Nutritional, Phytochemical, Antioxidant And Cytotoxic Potential Of' (2021), 48(3), Pp. 1–11.

[8]. Franzotti, E. M., Santos, C. V. F., Rodrigues, H. M. S. L., Mourão, R. H. V., Andrade, M. R., & Antoniolli, A. R. (2000). Anti-Inflammatory, Analgesic Activity And Acute Toxicity Of Sida Cordifolia L. (Malva-Branca). Journal Of Ethnopharmacology, 72(1–2), 273–277. Https://Doi.Org/10.1016/S0378-8741(00)00205-1

[9]. Gaidhani, K.A., Harwalkar, M. And Nirgude, P.S. (2014) 'World Journal Of Pharmaceutical Research SEED EXTRACTS', World Journal Of Pharmaceutical Research, 3(3), Pp. 5041–5048.

[10]. Hossein Hosseinzadeh; Mohammad Ramezani; Masoumeh Fadishei; Masoud Mahmoudi. (2002). Antinociceptive, Anti-Inflammatory And Acute Toxicity Effects Of Pistacia Vera L. Gum Extract In Mice And Rat. Phytomedicine, 1(4), 10–20.

[11]. Klančnik, A. Et Al. (2010) 'Evaluation Of Diffusion And Dilution Methods To Determine The Antibacterial Activity Of Plant Extracts', Journal Of Microbiological Methods, 81(2), Pp. 121–126. Available At: Https://Doi.Org/10.1016/J.Mimet.2010.02.004.

[12]. Nn, A. (2015) 'Medicinal & Aromatic Plants A Review On The Extraction Methods Use In Medicinal Plants , Principle , Strength And Limitation', 4(3), Pp. 3–8. Available At: Https://Doi.Org/10.4172/2167-0412.1000196.

[13]. P, V., M, S. And B, S. (2019) 'In Vitro Anti-Arthritic Activity Of Cissus Quadrangularis Stem Extract', Asian Journal Of Pharmaceutical And Clinical Research, 12(1), P. 250. Available At: Https://Doi.Org/10.22159/Ajpcr.2018.V12i1.27353.

[14]. Pal, S.K. And Shukla, Y. (2003) 'Herbal Medicine: Current Status And The Future', Asian Pacific Journal Of Cancer Prevention, 4(4), Pp. 281–288.

[15]. Paphassarang, S. Et Al. (1989) 'Triterpenic Glycosides From Polyscias Scutellaria', Phytochemistry, 28(5), Pp. 1539–1541. Available At: Https://Doi.Org/10.1016/S0031-9422(00)97786-0.

[16]. Ratnasooriya, W.D., Fernando, T.S.P. And Madubashini, P.P. (2008) 'In Vitro Thrombolytic Activity Of Sri Lankan Black Tea, Camellia Sinensis (L.) O. Kuntze', Journal Of The National Science Foundation Of Sri Lanka, 36(2), Pp. 179–181. Available At: Https://Doi.Org/10.4038/Jnsfsr.V36i2.151.

[17]. Sasaki, K., Abe, H. And Yoshizaki, F. (2002) 'In Vitro Antifungal Activity Of Naphthoquinone Derivatives', Biological And Pharmaceutical Bulletin, 25(5), Pp. 669–670. Available At: Https://Doi.Org/10.1248/Bpb.25.669.

[18]. Shaikh, J.R. (2020) 'Qualitative Tests For Preliminary Phytochemical Screening : An Overview', 8(2), Pp. 603–608.

[19]. Singh, J., Zaman, M. And Gupta, A.K. (2007) 'Evaluation Of Microdilution And Disk Diffusion Methods For Antifungal Susceptibility Testing Of Dermatophytes', Medical Mycology, 45(7), Pp. 595–602. Available At: Https://Doi.Org/10.1080/13693780701549364.

[20]. Suffredini, I.B. Et Al. (2006) 'Antibacterial And Cytotoxic Activity Of Brazilian Plant Extracts - Clusiaceae', Memorias Do Instituto Oswaldo Cruz, 101(3), Pp. 287–290. Available At: Https://Doi.Org/10.1590/S0074-02762006000300011.

[21]. Tuomilehto, J. (No Date) '2000 Ernst (2816)', 321, Pp. 395–396.

[22]. Umesh, M.K. Et Al. (2014) 'Evaluation Of In Vitro Anti-Thrombolytic Activity And Cytotoxicity Potential Of Typha Angustifolia L Leaves Extracts', International Journal Of Pharmacy And Pharmaceutical Sciences, 6(5), Pp. 81–85.

[23]. Vázquez, B. Et Al. (1996) 'Antiinflammatory Activity Of Extracts From Aloe Vera Gel', Journal Of Ethnopharmacology, 55(1), Pp. 69–75. Available At: Https://Doi.Org/10.1016/S0378-8741(96)01476-6.

[24]. Vickers, A. And Zollman, C. (1999) 'Herbal Medicine', 319(October), Pp. 16–19.

[25]. Wu, C. Et Al. (2015) 'A Comparison Of Volatile Fractions Obtained From Lonicera Macranthoides Via Different Extraction Processes : Ultrasound , Microwave , Soxhlet Extraction , Hydrodistillation , And Cold Maceration', Integrative Medicine Research, Pp. 1–7. Available At: Https://Doi.Org/10.1016/J.Imr.2015.06.001.

[26]. Zannah, F. Et Al. (2017) 'Phytochemical Screening Of Diplazium Esculentum As Medicinal Plant From Central Kalimantan, Indonesia', AIP Conference Proceedings, 1844. Available At: Https://Doi.Org/10.1063/1.4983439.