Anti-arthritic, anti-inflammatory, thrombolytic, membrane stabilizing, antifungal and cytotoxic activity of *Polysciasscutellaria* leaf extract: An in-vitro analysis

Abstract

The objective of this investigations was to evaluate in vitro anti-arthritic, anti-inflammatory, thrombolytic, membrane stabilizing, antifungal and cytotoxic activities from the methanolic leaf extract of*Polysciasscutellaria*. Primary evaluation ofmethanolic extract of *Polysciasscutellaria* leaf (MEPSL) was performed via phytochemical screening. Presence of Alkaloids, flavonoids, saponins, glycosides, carbohydrates and reducing sugars were observed by phytochemical screening, among other secondary compounds.Using in vitro methods of protein denaturation, the anti-arthritic and anti-inflammatory properties of MEPSL was investigated. The results showed that the extracts significantly slowed down arthritis and inflammation with the percent inhibition of 94.59% and 86.33% at the conc. of 1000µg/mL compared to the standard diclofenac sodium (98.19%) and acetyle salicylic acid (98.56%) at same concentration respectively. The thrombolytic activity of the extracts was additionally examined by clotlysis method, and the results showed that the ability to break up blood clots increased with the amount of extract used with the value of 97.32% which is very significant compared to the standard streptokinase which showed clotlysis of 91.304%. MEPSL was also showed action that stabilized membranes by using heat induced hemolysis method, which could be helpful in treating conditions like bleeding and swelling with the percent of protection value of 58.87% when compared to standard diclofenac sodium with the percent of protection value 73.63%. Antifungal action was also seen, which shows that it could be used to treat diseases caused by fungi with the zone of inhibition 7-36 mm varying on the type of fungi. Lastly, in vitro method was used to investigate the extracts' damaging effect on *Artemia salina* by using shrimp lethality assay. The results showed significant cytotoxicity with the LC_{50} value of 1.057µg/mL compared to the standard vincristine sulphate (LC_{50} value of 0.608µg/mL). To sum up, it is clear that the phytochemical found inthis plant can be used for wide range of drug discovery field due to its potent pharmacological actions.

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

1. 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 notcontain any adverse effect. Besides, many of the few potent medicines were plant derived. Examples includemorphine (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).

Polysciasscutellaria(Burm.f.) Fosberg also known as shield aralia, plum aralia which is a small shrub or bush reaching 2-6 meters in height.The genus Polyscias of family Araliaceaecomprises about 116 species that are widely used for ornamental purposes, some with potential medicinal value. It is commonly growing in pacific country (Vanuatu). It has significant anti-inflammatory properties(Paphassarang *et al.*, 1989).Traditionally, this plant was used to treat breast inflammation, wounds, urinary tract problem and body odor.

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

2. MATERIALS AND METHODS

2.1 Plant Materials

The sample plant *Polysciasscutellaria* (Burm.f.) Fosbergwas collected in November, 2022 from Ramna Park, MoulanaBhashani Road, Dhaka, Bangladesh. Then the plant (accession number: DACB 88046) was precisely recognized by the professionals at the Bangladesh National Herbarium in Mirpur, Dhaka. In the mean time, plant's leaves had been stored and dried in shade and powder was made from these dried leaves.

2.2 Reagents

Methanol, concentrated H_2SO_4, Diluted HCl acid, acetic acid and NaOH was supplied by Sigma Chemical Co.,USA.FromPolysciences, 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.

2.3 Preparation of Plant Extract

The extraction of plant was obtained by using cold maceration method(Nn, 2015). About 80g powder of *Polysciasscutellaria* leaf was soaked in 600mL of methanol for 10days 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 7days. After drying, overall weight of 16.15g of leaf extract was obtained.

2.4 Phytochemical Screening Test

Many therapeutic characteristics of the plants are obtained from its chemical constituents(Shaikh, 2020). Freshly prepared MEPSL was screened qualitatively for the presence of phytochemicals such as alkaloids, carbohydrates, saponins, glycosides, reducing sugar, flavonoids, tannins and steroids.

2.5 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 0.45mL and MEPSL of 0.05mLare together formed 0.5mL of test solution and as a standard drug, 0.05mL of Diclofenac sodium were used. MEPSL 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 2.5mL phosphate buffer was added after cooling the solution. Finally, at a wavelength of 416nm the absorbance of the solutions was taken by using UV-Visible spectrometer. Here, 0.05mL 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 as follows:

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

Here, OD means optical density.

2.6 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 5mL of reaction mixture containing 2.8mL of phosphate buffered saline (PBS, pH 6.4), 0.2mL of egg albumin (from a hen's egg), and 2mL of MEPSL. Double-distilled water was used at equal amount for control group. At 70^{o}C, the mixtures were heated for 5 minutes after incubating the mixture at (37±2)^{o}C, using Biological Oxygen Demand(BOD) incubator for a time period of 15minutes. After cooling, absorbance of the mixtures was taken at 660nm. 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 as follows:

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

2.7 In VitroThrombolyticTest

2.7.1 Blood Sample:

4mL of venous blood was drawn from healthy human volunteers (n=15), whom had never consumedblood 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 500µL of fresh blood.

2.7.2 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 irritationto the piercing area and the time period for blood collection.

2.7.3 Clotlysis method:

The method used for determining the percentage of clotlysiswas obtained previously published research paper(Umesh *et al.*, 2014). In short, 2.5mL of fresh blood was filled in 15 discrete pre-weighed sterile microcentrifuge tubes (0.5mL/tube) and at 37^{o}C,it was incubated for 45minutes. 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 100µL of MEPSL was added to each microcentrifuge tube which contain pre-weighted clot. By adding 2.5mL of PBS, lyophilized streptokinase vial was reconstituted and was mixed properly. In the volume of 100µL 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 37^{o}C for 90minutes. 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 andthe equation used for this determination as follows:

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

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

2.8 Membrane Stabilizing Activity Test

2.8.1 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).

2.8.2 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 (1mL, pH 7.4), 0.36% hyposaline (2mL), 10% v/v HRBC suspension (0.5mL) with plant extracts (0.5mL) 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^{o}C for 30min and centrifugation respectively. Spectrophotometer at 560nm 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]

2.9 Antifungal Susceptibility Test

2.9.1 Fungal strains:

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

2.9.2 Disc Diffusion Method:

Antifungal activity of MEPSL 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 1mL culture of each fungus was spread uniformly throughout the medium. Sterile filter paper disc of 6mm in diameter was used and this disc was saturated with diluted MEPSL of 10µL, setting on the top of each agar plate. In this test, MEPSL was taken in several concentration (300, 500, 700µg/mL). Then the plates were put on the incubator for next 24hours. Griseofulvin containing disc was used as an antifungal agent for positive control, while methanol containing disc was used for negative control. After 24hours, based on the size of inhibition zone surrounding the disc, measured in mm, antifungal activity was determined(Singh, Zaman and Gupta, 2007).

2.10 In Vitro Cytotoxic Activity Test

Cytotoxic activity of MEPSL was investigated using the brine shrimp lethality test, a standard bioassay for screening bioactive compound (Riaz *et al*, 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 MEPSL was prepared by dissolving it in a dimethyl sulfoxide solution that cannot be more than 50µL per 5mL. Then artificial seawater was mixed up to 5mL 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 24hours to see how many nauplii had survived. By utilizing a logarithmic plot of concentration against mortality rate, LC_{50} was calculated.

2.11 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.

3. RESULTS

3.1 Phytochemical Screening Test:

From this screening test, it was identified that alkaloids, carbohydrates, saponins, glycosides, reducing sugars, and flavonoids were present, wheareas, tannins and steroids were absent in MEPSL (Table 1.)

Table 1. Qualitative phytochemical analysis of MEPSL.

Phytochemical constituent	MEPSL
Alkaloid	++
Carbohydrate	++
Saponin	++
Glycoside	+
Reducing Sugar	+
Flavonoid	+
Tannin	-
Steroid	-

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

3.2 In Vitro Anti-Arthritic Test:

Denaturation of BSA property compared to the standard drug has been shown in Table 2 and Figure 1.

Table 2. 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
MEPSL	62.5	83.78
	125	84.68
	250	87.38
	500	93.69
	1000	94.59

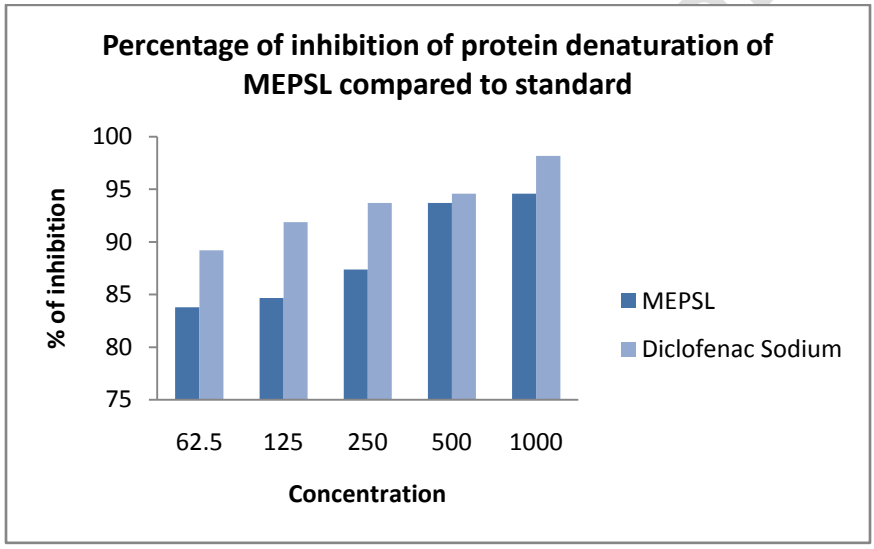

Fig 1. Percentage of inhibition of MEPSL compared to standard

3.3In Vitro Anti-Inflammatory Test:

The percentage of proteinase inhibition carried out by MEPSL shows a dose dependent rise which is in moderate level compared to the standard and shown in Table 3 and Figure 2.

Table 3. 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
MEPSL	62.5	79.86
	125	80.58
	250	82.01
	500	82.73
	1000	86.33

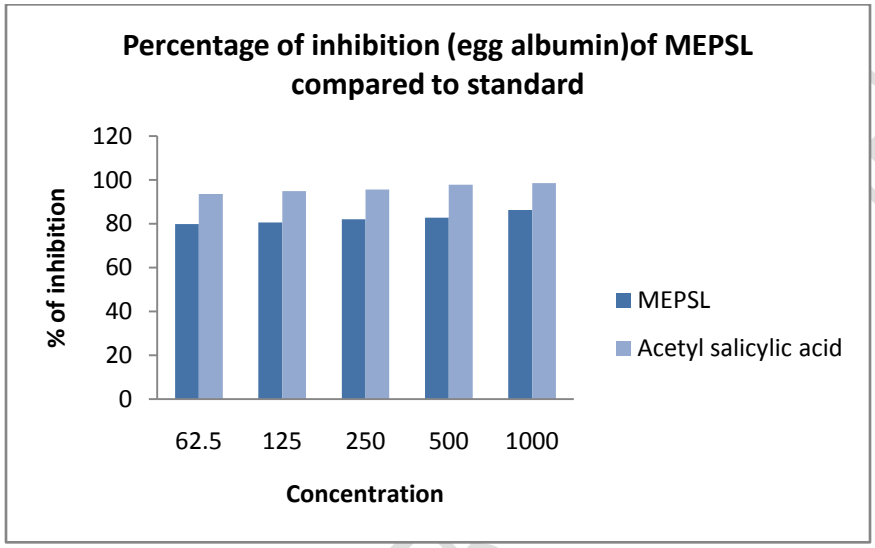

Fig 2. Percentage of inhibition of MEPSL compared to standard using egg albumin.

3.4In VitroThrombolyticTest:

Thrombolytic activity of MEPSL is very significant than compared to standard drug (Table 4). So, it can be assumed that MEPSL can be used as a drug like plasmin which can reduce blood clots.

Table 4. Percentage of clot lysis, n=15 (mean value)

Sample	% of clot lysis
Negative control	7.296
Streptokinase	91.304
MEPSL	97.32

3.5Membrane Stabilizing Activity Test:

Percentage of hemolysis and protection of MEPSL compared to the standard is measured in this test which is deliberated in Table 5.

Table 5. Percentage of hemolysis of RBC by MEPSL extract

Sample	% of hemolysis	% of protection
Diclofenac Sodium	26.36	73.63
MEPSL	41.18	58.87

3.6Antifungal Susceptibility Test:

A moderate antifungal activity has obtained compared to the standard drug which is demonstrated in Table 6.

Table 6. 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**

3.7 In Vitro Cytotoxic Activity Test:

In **Table 7.** The cytoxic activity of MEPSL to brine shrimp nauplii is summarized and standard calibration curve of standard and MEPSL, which shows the effect of both on brine shrimp nauplii, illustrated in **Figure 3** and **Figure 4** respectively.

Table 7. Brine Shrimp Assay (Mortality %, LC_{50} value)

Sample	Concentration (C) (µg/mL)	Mortality %	LC_{50} value

Vincristine Sulphate	1.95 3.91 7.81 15.325 31.25 62.5 125 250 500	40 40 60 70 80 90 90 100 100	0.608
MEPSL	1.95 3.91 7.81 15.625 31.25 62.5 125 250 500	20 30 40 60 70 80 90 100 100	1.057

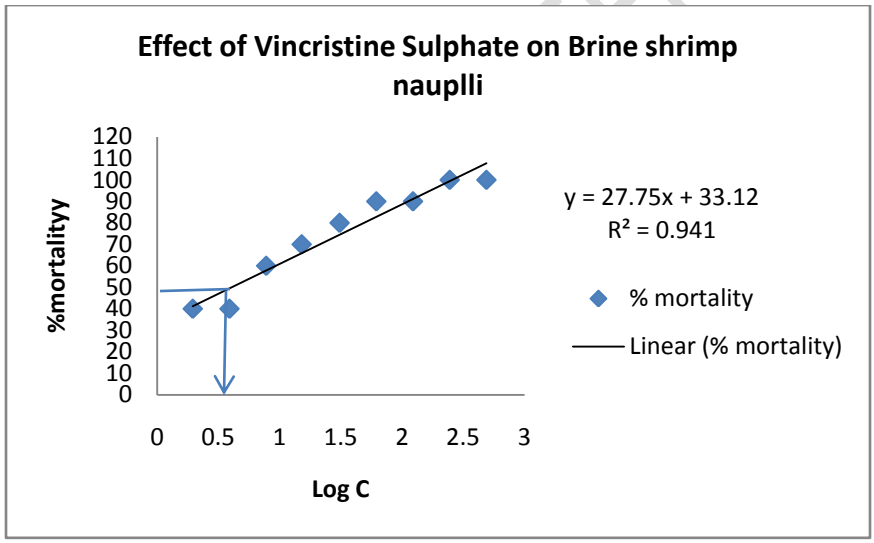

Fig 3. Cytotoxic activity of Vincristine Sulphate on Brine shrimp nauplii

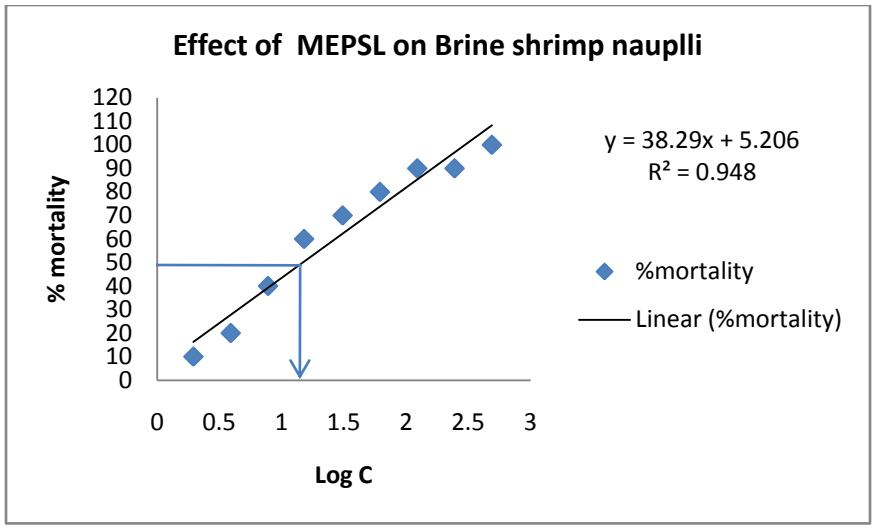

Fig 4. Cytotoxic activity of MEPSL on Brine shrimp nauplii

4. 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 (Riaz *et al*, 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 1000µg/mL, which is very close when compared to Diclofenac sodium's value of 98.19% in 1000µg/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 1000µg/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 1000µg/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 *Polysciasscutellaria* 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 *Polysciasscutellaria* leaf. It can be said that flavonoids are responsible for this type activity. Hence, *Polysciasscutellaria* 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 500µg/mLand conversely lowest at a concentration of 1.95µg/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).

5.0 CONCLUSION

As in many other countries, *Polysciasscutellaria* can be found growing wild in Bangladesh. The above description makes it very evident that *Polysciasscutellaria* is rich in phytochemicals and serves several pharmacological purposes. Previously, it was hypothesized that the crude methanolic extract of *Polysciasscutellaria* 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 *Polysciasscutellaria* 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.

8.0 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.

References:

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

Riaz Iqra , Yamin Bibi, Nabeela Ahmad, Sobia Nisa, A. Q. (2021). Evaluation of nutritional, phytochemical, antioxidant and cytotoxic potential of. Kuwait Journal of Science, 48(3), 1–11.

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.

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.

Shaikh, J.R. (2020) 'Qualitative tests for preliminary phytochemical screening : An overview',

8(2), pp. 603–608.

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.

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.

Tuomilehto, J. (no date) '2000 Ernst (2816)', 321, pp. 395–396.

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.

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.

Vickers, A. and Zollman, C. (1999) 'Herbal medicine', 319(October), pp. 16–19.

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.

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.