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RESEARCH ARTICLE

Phytochemical Analysis and *in vitro* Evaluation of Anti-Inflammatory and Thrombolytic Activities Using Fractional Crude Extracts Derived from Six Medicinal Plants from Chittagong Hill Tracts of Bangladesh

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Abstract

The ethnic people of Chittagong Hill Tracts in the southern region of Bangladesh use *Ardisia paniculata, Ardisia solanaceae, Argyreia capitiformis, Diplazium splendens, Ixora finlaysoniana,* and *Pouzolzia sanguinea* to alleviate fever, diarrhea, cough, dysentery, inflammation, insomnia, and analgesia. However, there is no scientific evidence regarding the bioactivity of these plants. This study aimed to explore phytochemicals, *in vitro* anti-inflammatory and thrombolytic activities using phytochemical qualitative analysis, protein denaturation and clot disruption methods. The extracts were prepared using a Soxhlet extractor with methanol and further fractionated with ethanol, chloroform, and n-hexane. The phytochemical evaluation indicates alkaloids, glycosides, amides, phenols and vitamins present in ethanol fractions of all medicinal plants. The anti-inflammatory activity of the ethanol extract of *P. sanguinea* showed better activity compared to other studied medicinal plants. The ethanol fraction exhibited the highest inhibition of protein denaturation (62.52%), followed by n-hexane (59.67%) and chloroform (49.43%) at 160 µg/mL of *P. sanguinea* in *in vitro* anti-inflammatory test. The IC_{50} values for *P. sanguinea* were 114.50 µg/mL, 129.09 µg/mL, and 150.17 µg/mL, which were lower than other medicinal plants in the anti-inflammatory test. Acetylsalicylic acid, used as a positive control, demonstrated 82.29% effectiveness at similar dosages, with an IC_{50} value of 79.39 µg/mL. The chloroform fraction of *I. finlaysoniana* showed moderate clot lysis (55.43%) compared to positive control streptokinase (80.31%) and other medicinal plants. Furthermore, this study revealed that the ethanol fraction of *P. sanguinea* leaves is more potent and it showed anti-inflammatory activity, while chloroform fraction of *I. finlaysoniana* leaves showed more clot lysis activities compared to other studied medicinal plants. This study opens new insights into pharmacological potential of Bangladeshi medicinal plants, which might be useful for developing cost-effective, plant -based medicine to protect chronic inflammation and cardiovascular diseases.

Keywords: Medicinal Plants, Phytochemicals, Anti-Inflammatory, Clot-Lysis.

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Introduction

Bangladesh, a tropical nation with exceptionally fertile soil, hosts a remarkable array of medicinal plants vital for health and wellness. The Chittagong Hill Tracts (CHTs) are the southern region of Bangladesh, consisting of three hill districts, Bandarban, Rangamati, and Khagrachari, which encompass about ten percent of Bangladesh's total geographical area (Chowdhury et al. 2008). This region contains one-third of the nation's blooming plants and has significant floral biodiversity. Approximately thirteen ethnic groupings reside in this hilly section of the nation. The ethnic tribes of the hill areas consistently seek indigenous plants for diverse applications, therefore amassing significant knowledge about the use of wild flora throughout time. A total of 204 species from 160 genera and 76 families used by communities in the CHTs to treat about 197 diseases/ailments were documented. Leaves were the most used component (145 species), followed by roots (119 species) and stems (53 species) (Faruque et al. 2018). Medicinal plants play a crucial role in the pharmaceutical industry, offering alternative treatments to address the limitations of synthetic medications (Habib et al. 2023). Several medicinal plants are utilized in Chittagong and CHT; however, there is limited information regarding their

medicinal properties. Among these, *Ardisia paniculata* Roxb. is utilized for the treatment of analgesia, inflammation, clot lysis and sedation, with leaf extracts given orally three times daily. *Ardisia solanaceae* Roxb is utilized in the treatment of boils, skin infections through the application of a paste made from fresh leaves to the affected area (Amin et al. 2015). *Argyreia capitiformis* is utilized in the treatment of leg bruises; a paste derived from its leaves is applied to the affected area. *Diplazium splendens* is utilized in the management of fever, inflammation and headaches. *Ixora finlaysoniana* has been utilized to treat certain bacterial infections as well as inflammation. *Pouzolzia sanguinea* is utilized in the management of flatulence, with leaf extracts given orally with sugar twice daily. The fractionated extracts of *P. sanguinea* exhibited anti-inflammatory, analgesic, antipyretic, and antioxidant properties (Ahsan et al. 2021). Three derivatives of dihydrostilbene (1-3) and five neolignans (4-8) were isolated from the ethyl acetate soluble fraction of *P. sanguinea* (Ahsan et al. 2025). We thought these six medicinal plants may have anti-inflammatory and clot lysis activities, which could be a new approach for phytomedicine production. The principle aims to evaluate *in vitro* from selected six indigenous medicinal plants- *A. paniculata*, *A. solanaceae*, *A. capitiformis*, *D. splendens*, *I. finlaysoniana*, and *P. sanguinea* based on their traditional uses, especially anti-inflammatory and clot lysis activities in the tribal region of Chittagong Hill Tracts.

Materials and Methods

Chemicals and solvents

The ethanol, methanol, chloroform, and n-hexane were purchased from Merck, Germany (analytical grade). Standard drugs were collected from various manufacturers: acetylsalicylic acid (75 mg, Cardopin, BEXIMCO Bangladesh Ltd.), Streptokinase (STK 1500000 I.U, Incepta Pharmaceuticals Ltd.). All standard marketed drugs were used as stock solutions for particular tests without further purification.

Selection and identification of plant species

The leaves of the mentioned plants were selected based on its medicinal uses. The leaves were washed with tap water to eliminate dust, sediments, and parasites. Taxonomic identification of these medicinal plants was identified by Bangladesh Forest Research Institute Herbarium (BFRIH) in Chattogram, receiving voucher Nos, SA: 5442020, IA: 4952020, SA: 5462020, SA: 5512021, SA: 5522021 and SA: 5102020.

Collection and garbling

This plant's leaves were obtained from multiple regions in Chattogram between January 2023 and January 2024. The extraneous, undesired substances from the plant material were removed at two stages of processing of it. After collecting them, the rotten leaves were first removed by hand. The leaves were treated with tap water to eliminate soil, dust, sediments, and parasites by washing those. To prevent the formation of germs and mold, it is customary to dry plant materials on a plate at room temperature with enough ventilation for three weeks in the shade. After drying, a high-limit pounding grinding machine (Xiangrong, China) was utilized to crush of those plant leaves to make fine powder and then sieving was done. Then, it was pressed into a plastic compartment that is hermetically sealed and marked appropriately. Subsequently, it was stored in a dull, cool, and dry area until it was necessary for the next procedure.

Soxhlet extraction or hot continuous extraction

For hot extraction, approximately 500 g of leaves powder of the *A. paniculata* was subjected with 2000 mL of methanol (98.85%) and in a Soxhlet apparatus (SE-250~2000, China, Pyrex, 3.3 Glass). After collecting the material, filtering it with filter paper (Whatman No. 1), and evaporating it with a rotary evaporator (Wincom, RE 52A, China) at temperatures below 50°C. The solvent was evaporated, resulting in a gummy concentrate that was labelled as hot methanol crude extracts (Kupchan et al. 1973). Following the same process, 500 g leaves powder of the *A. solanacea* with 2000 mL of methanol, 400 g leaves powder of the *A. capitiformis* with 1800 mL of methanol, 400 g leaves powder of the *D. splendens* with 1800 mL of methanol, 400 g leaves powder of the *I. finlaysoniana* with 1500 mL of methanol and 500 g leaves powder of the *P. sanguinea* with 2000 mL of methanol to produce a gummy concentrate of methanol crude extracts.

Fractionation with different polar solvents

The crude methanol extract (20 g) of *A. paniculata* was dissolved in ethanol with 10 mL double distilled water (DDW) and then fractionated using a fractionating column (HCl 1000, China, Boro 3.3 glass) with n-hexane and subsequently with chloroform (Kupchan et al. 1973). Until 150 mL of n-hexane was used, the solution was fractionated using 50 mL of solvent every time, and the mixture was allowed to stand after vigorous shaking each time. Solvent layers were separated and decanted. The extract that was left was utilized as an ethanol fraction. The extracts that were obtained were collected, filtered, and evaporated the temperature below 50°C. The solvent's

evaporation resulted in the creation of a gummy concentrate. After being weighed, the gummy concentrates were placed in an air-tight container that was properly labeled and clean, and stored at 4°C. The extracts were weighed separately with the help of an electronic and analytical balance (AS 202. R2 Plus Radwag, Poland). Then the yield was determined by the following equation.

$$\text{Yield percentage} = \frac{\text{Dry weight of extract}}{\text{Total weight of extract}} \times 100$$

Phytochemical analysis

Qualitative tests were conducted to ascertain the chemical composition of fractional extracts of *A. paniculata*, as outlined in the Practical Pharmacognosy Textbook (Ghani 2005). The concentration of color or the production of precipitation is ascertained using phytochemical screening. The qualitative outcome was assessed using a positive '+' sign to indicate the presence and a negative '-' sign to denote the absence of phytochemicals (Larayetan et al. 2017).

Test for alkaloids

A minimal quantity of the sample solution (2 mL) was diluted in hydrochloric acid and then filtered. The filtrate solution was treated with Drafendroff's reagents. The emergence of a red precipitate indicated the existence of alkaloids.

Test for glycosides

0.5 mL glacial acetic acid was added to a 2 mL sample solution, and then a few drops of ferric chloride and 0.5 mL of concentrated sulfuric acid- a brown ring formed between two layers indicating cardiac glycosides (Keller-Killing test).

Test for steroids

A small quantity of sample solution was dissolved in chloroform, followed by the addition of 1-2 drops of sulphuric acid and 2-3 drops of acetic anhydride. The bright green color at the interface of the two layers indicated the presence of steroids (Liebermann-Burchard test).

Test for tannins

A 2-3 mL sample solution was placed in a test tube, followed by adding a 5% ferric chloride solution. The dark blue hue and precipitate suggested the presence of tannins.

Test for flavonoids

A small sample was dissolved in an alcoholic solution, and 2-3 drops of conc. H_2SO_4 added. The rapid emergence of red color signifies the presence of flavonoids.

Test for terpinoids

5 mL of Salkowski's reagent were added to a 2 mL sample solution. The reddish-brown color indicated the existence of terpenoids.

Test for sapnonins

Approximately 0.5 mL of the sample solution was mixed with 5 mL of distilled water. The foam formation on the liquid layer indicated the presence of saponins.

Test for amides

A small sample was dissolved in 5 mL of a 20% NaOH solution and heated to boiling for 15 mins. The release of NH_3 gas, which causes red litmus paper to turn blue, signified the presence of amides.

Test for phenols

2 mL of the sample solution were combined with 2 mL of distilled water, followed by the addition of a 10% ferric chloride solution. The solution's bluish-black color signified the presence of phenols.

Test for vitamin C

1 mL of the sample solution was treated with diphenyl hydrazine, followed by the addition of conc. H_2SO_4 yellow color signified the existence of vitamin C.

Test for quinone

10 mL of extract were dissolved in isopropyl alcohol, followed by the addition of one drop of concentrated sulphuric acid. The red color indicated the presence of quinone.

In vitro anti-inflammatory assay

The reaction mixture contained 3 mL of 5% egg albumin solution and 3 mL of fractional crude extracts to prepare final concentrations of 10, 20, 40, 80, and 160 µg/mL. A similar volume was used for standard (acetylsalicylic acid) as a positive control respectively. Distilled water used as a control. To maintain consistency, all reaction mixtures' pH (6.2±6.5) was carefully adjusted with 0.1N HCl. The mixtures were incubated at 37±2°C in a BOD incubator (Labline Technologies) for 15 mins before heating at 70ºC for 5 mins. After cooling, their absorbance was measured at 660 nm. The percent of inhibition was calculated by following equation.

$$\% \text{ Inhibition protein denaturation} = \frac{\text{Mean absorbance [control} - \text{sample]}}{\text{Mean absorbance control}} \times 100$$

In vitro thrombolytic assay

Venous blood samples were collected from twenty healthy male and female participants aged 20 to 30, ensuring the validity of the experiment. The experiment was replicated on various days using blood samples from healthy participants, both male and female, who were not using contraceptives or anticoagulants. Approximately 500 µL of blood was added to each pre-weighed microcentrifuge tube to facilitate clot formation, with each tube distinctly labeled with a unique number. The thrombolytic activity test was conducted using the technique with minor modifications. 10 mL of venous blood, collected with utmost care from healthy volunteers, were allocated into several groups (each group, n = 5): 18 for fractional extracts (six medicinal plants), one for a reference standard (streptokinase) as a positive control, and one for negative control, all contained in pre-weighed sterile microcentrifuge tubes (500 µL per tube) and incubated at 37°C for 45 mins. Following clot formation, serum was removed without disrupting the clot, and each tube holding the clot was reweighed to ascertain the clot weight (Clot weight = Weight of tube with clot – Weight of empty tube).

The positive control tubes received streptokinase (50 µg/10 µL), whereas the experimental groups were administered their corresponding fractional extracts (500 µg/100 µL). Ethanol was introduced into the control tubes. Subsequently, all tubes were incubated at 37°C for 90 mins and monitored for clot lysis. Following incubation, the released fluid was removed, and the tubes were reweighed to assess the weight variation after clot disruption. The percentage of clot lysis was determined using the following formula.

$$\% \text{ Clot lysis} = \frac{\text{Mean weight of clot before treatment}}{\text{Mean weight of clot after treatment}} \times 100$$

Statistical analysis

The data is presented as mean ± standard error of the mean (SEM). Statistical analysis was conducted utilizing one-way ANOVA, followed by the Tukey post hoc test, within Graphpad Prism 5 software to compare results against the control and among the various groups. P-values of $p<0.001$ indicate statistical significance.

Results and Discussion

Phytochemical analysis of six medicinal crude extracts

Results showed that ethanol, chloroform and n-hexane extracts of six medicinal plants in this study showed alkaloids, glycosides, amides and vitamin C. Steroids were found in all the plant extracts except *D. splendens.* Tannins were present in *A. paniculata, I. finlaysoniana* and *P. sanguinea* extracts. The entire fraction, flavonoids, terpenoids and phenols were present in only two medicinal plants i.e. *A. paniculata* and *P. sanguinea* extracts. Quinones were absent in *D. splendens*. Effective phytochemicals identified from *A. paniculata* and *P. sanguinea* fractional extracts (Table 1).

Table 1: Qualitative phytochemicals of fractional extracts of six medicinal plants.

Phytochemical constituents		Alkaloids	Glycosides	Steroids	Tannins	Flavonoids	Terpenoids	Saponins	Amides	Phenols	Vitamin C	Quinones
Medicinal plants	Fractional extracts											
A. paniculata	Ethanol	+	+	+	+	+	+	+	+	+	+	+
	Chloroform	+	+	-	+	+	+	+	+	+	+	+
	n-hexane	+	+	+	+	+	+	+	+	+	+	-
A. solanaceae	Ethanol	+	+	+	-	-	+	+	+	+	+	+
	Chloroform	+	+	+	+	-	-	+	+	-	+	-
	n-hexane	+	+	+	+	+	-	+	+	-	+	+
A. capitiformis	Ethanol	+	+	+	-	-	-	-	+	-	+	-
	Chloroform	+	+	+	-	+	+	+	+	+	+	+
	n-hexane	+	+	+	-	+	+	+	+	+	+	+
D. splendens	Ethanol	+	+	-	-	-	-	-	+	+	+	-
	Chloroform	+	+	-	+	-	-	-	+	-	+	-
	n-hexane	+	+	-	+	-	-	-	+	-	+	-
I. finlaysoniana	Ethanol	+	+	+	+	+	+	+	+	+	+	+
	Chloroform	+	+	-	+	+	+	+	+	+	+	+
	n-hexane	+	+	+	+	-	+	+	+	-	+	+
P. sanguinea	Ethanol	+	+	+	+	+	+	+	+	+	+	+
	Chloroform	+	+	-	+	+	+	+	+	+	+	+
	n-hexane	+	+	+	+	+	+	+	+	+	+	+

'+' sign indicates presence and '–' sign indicates absence in the test.

In vitro anti-inflammatory activity of fractional extracts from six medicinal plants

The efficacy of fractional extracts from six medicinal plants was evaluated, revealing promising findings on the percentage inhibition of protein denaturation. The ethanol fraction emerged as the most potent, demonstrating a significant 62.52% inhibition, followed by the n-hexane fraction at 59.67% and the chloroform fraction at 49.43%, all measured at 160 µg/mL of *P. sanguinea*. The IC_{50} values for *P. sanguinea* were 114.50 µg/mL, 129.09 µg/mL, and 150.17 µg/mL, respectively, compared to other medicinal plant extracts (Table 2). Acetylsalicylic acid, a positive control in the field, showed an efficacy of 82.29% at comparable doses, with an IC_{50} value of 79.39 µg/mL. The observed linearity in the n-hexane fraction of *P. sanguinea* is ideal, characterized by an R^2 value of 0.99 and the linear equation, $y = 0.3673x + 2.582$. *A. paniculata* and *P. sanguinea* demonstrated significant anti-inflammatory activity ($p<0.001$) compared to the standard acetylsalicylic acid (Table 2). Conversely, all fractions of *A. capitiformis* and *D. splendens* exhibited mild anti-inflammatory activities. The dose-dependent effect on protein denaturation observed in all test groups further underscores the potential impact of these findings on future research and drug development (Fig. 1).

Table 2: *In vitro* anti-inflammatory activity of six selected distinct medicinal fractional crude leaf extracts by inhibition of protein denaturation methods.

Plant extracts	% Mean inhibition in protein denaturation (µg/mL) Mean ± SEM					IC_{50}(µg/mL) /R^2
	10	20	40	80	160	
ASA(S)	15.19±0.02	26.94±0.06	38.64±0.02	57.68±0.03	82.29±0.04	79.39/0.92
EEAP	6.95±0.06	13.38±0.05	25.47±0.08	36.44±0.02	56.47±0.01	163.70/0.95
CEAP	4.01±0.02	8.85±0.05	20.51±0.07	31.82±0.06	43.99±0.04	167.15/0.93
HEAP	3.32±0.06	6.86±0.06	13.73±0.05	21.15±0.03	33.98±0.04	228.54/0.97
EEAS	2.76±0.01	6.73±0.04	17.78±0.04	29.18±0.03	39.16±0.02	187.54/0.92
CEAS	3.92±0.03	7.55±0.01	15.19±0.02	23.27±0.02	37.91±0.04	204.41/0.97
HEAS	5.09±0.02	11.57±0.02	17.74±0.05	21.63±0.01	34.67±0.01	227.65/0.91
EEAC	3.41±0.02	8.59±0.02	16.96±0.04	27.28±0.03	47.15±0.02	164.11/0.98
CEAC	2.41±0.02	7.55±0.02	12.61±0.01	29.01±0.03	42.61±0.03	176.94/0.96
HEAC	2.07±0.02	5.91±0.02	11.39±0.03	26.21±0.04	38.77±0.02	194.90/0.97
EEDS	1.25±0.03	3.23±0.05	7.16±0.01	13.08±0.06	24.91±0.06	318.12/0.99
CEDS	1.07±0.04	2.63±0.02	6.13±0.02	12.30±0.01	23.53±0.02	334.45/0.99
HEDS	1.03±0.05	2.20±0.05	5.52±0.02	11.74±0.03	20.55±0.03	379.38/0.99
EEIF	4.40±0.05	7.81±0.05	16.71±0.02	24.74±0.03	41.32±0.03	227.65/0.97
CEIF	5.48±0.04	9.11±0.01	16.62±0.02	21.76±0.01	38.29±0.03	206.83/0.96
HEIF	5.91±0.01	8.51±0.02	16.92±0.01	20.37±0.01	37.78±0.04	212.02/0.96
EEPS	9.88±0.01	17.70±0.06	30.22±0.02	41.53±0.05	62.52±0.03	114.50/0.93
CEPS	7.46±0.02	10.06±0.04	19.21±0.01	34.62±0.02	49.43±0.04	150.17/0.95
HEPS	6.47±0.01	09.71±0.07	19.04±0.02	34.45±0.01	59.67±0.01	129.09/0.99

Values are expressed as percentage ± SEM (n = 3) each group; ASA: Acetysalicylic acid (positive control) for standard; EEAP, CEAP, HEAP, EEAS, CEAS, HEAS, EEAC, CEAC, HEAC, EEDS, CEDS, HEDS, CEIF, CEIF, HEIF, EEPS, CEPS, HEPS= ethanol, chloroform, n-hexane extract *A. paniculata*, *A. solanaceae*, *A. capitiformis, D. splendens*, *I. finlaysoniana*. Analysis using one-way ANOVA (graph pad prism software) followed by Tukey post hoc test compared to control. The P-value *p<0.001* significant.

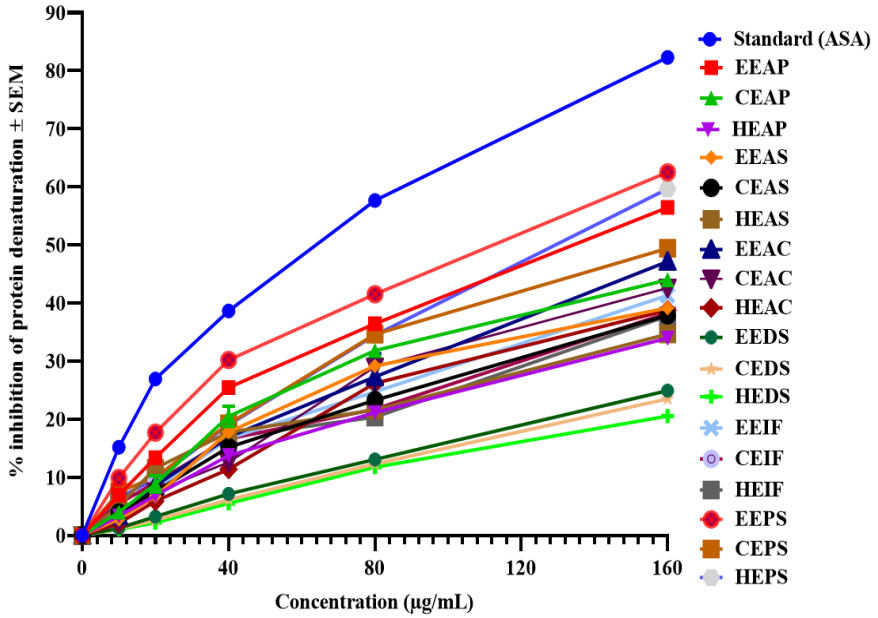

Fig. 1: The percentage of six medicinal fractional crude extracts that inhibit protein denaturation was evaluated.

Our standard positive control, acetylsalicylic acid (ASA), displayed the highest anti-inflammatory properties. This was followed by the ethanol extract of *P. sanguinea* (EEPS), the n-hexane extract of *P. sanguinea* (HEPS), and the ethanol extract of *A. paniculata* (EEAP). The p-value *p<0.001*, significant (one way ANOVA followed by Tukey post hoc test).

In vitro thrombolytic activity of fractional extracts from six medicinal plants

This study aimed to explore cardioprotective medicines from plant reserves by analyzing the thrombolytic (clot lysis) activity of fractional crude extracts from six different medicinal plants. The clot lysis percentage of the chloroform fraction of *I. finlaysoniana* was 55.43%, the highest percentage of any other fractional extracts of medicinal plants. This was followed by the clot lysis percentage of the n-hexane fraction, which was 55.17%, and then the clot lysis from the ethanol fraction of *I. finlaysoniana* exhibited 53.96%. Compared to the normal streptokinase, which displayed clot lysis was 80.31%, the ethanol fraction of *P. sanguinea* had a clot lysis rate of 52.40%. In contrast, the percentage of clot lysis observed with distilled water as a negative control was negligible at 3.19%. All fractional extracts of *I. finlaysoniana* and *P. sanguinea*, including ethanol, chloroform, and n-hexane fractions, demonstrated significant (*p<0.001*) thrombolytic activity, which confirms their cardiotonic properties. The unique cardioprotective qualities of *I. finalaysonian* and *P. sanguinea* require further investigation. Conversely, the ethanol fraction of *A. solanaceae* had the lowest proportion of clot lysis, at only 12.07%. The n-hexane fractions of *A. paniculata*, *A. solanaceae*, *D. splendens* and *A. capitiformis* showed almost identical percentages (Table 3). Fig. 2 illustrates the comparative thrombolytic activity of ethanol, chloroform, and n-hexane fractional extracts from *A. paniculata, A. solanaceae, A. capitiformis, D. splendens, I. finlaysoniana*, and *P. sanguinea*.

Table 3: *In vitro* clot lysis activities of six medicinal plant species.

Medicinal plants	Fractional extracts	Mean wt. of clot (gm), A	Mean wt. of lysis clot (gm), B	Percentage of lysis (%) = (B*100) /A
Control	Solvent	0.720±0.011	0.023±0.014	3.191
+Ve Cont.	Streptokinase	0.791±0.012	0.659±0.032	80.312
A. paniculata	Ethanol	0.826±0.009	0.199±0.030	24.153
	Chloroform	1.073±0.081	0.197±0.074	18.328
	n-hexane	1.063±0.064	0.178±0.070	16.771
A. solanaceae	Ethanol	1.090±0.029	0.132±0.053	12.075
	Chloroform	1.003±0.006	0.165±0.008	16.451
	n-hexane	1.023±0.024	0.191±0.027	18.631
A. capitiformis	Ethanol	0.692±0.017	0.162±0.014	23.339
	Chloroform	0.664±0.038	0.161±0.060	24.297
	n-hexane	1.002±0.005	0.146±0.012	14.608
D. splendens	Ethanol	1.128±0.006	0.405±0.010	35.893
	Chloroform	1.117±0.002	0.264±0.012	23.627
	n-hexane	1.003±0.019	0.199±0.078	19.847
I. finlaysoniana	Ethanol	1.081±0.006	0.583±0.001	53.962
	Chloroform	1.042±0.003	0.577±0.031	55.438
	n-hexane	1.054±0.004	0.581±0.003	55.172
P. sanguinea	Ethanol	1.012±0.026	0.530±0.034	52.404
	Chloroform	1.075±0.007	0.476±0.017	44.062
	n-hexane	1.009±0.004	0.443±0.017	43.923

Values are expressed as percentage ± SEM (n = 5) each group; solvent = distilled water as a control; positive (+Ve) control= streptokinase. Analysis using mean one-way ANOVA (Graph pad prism software) followed by Tukey post hoc test. The p-value *p<0.001* significant compared to control.

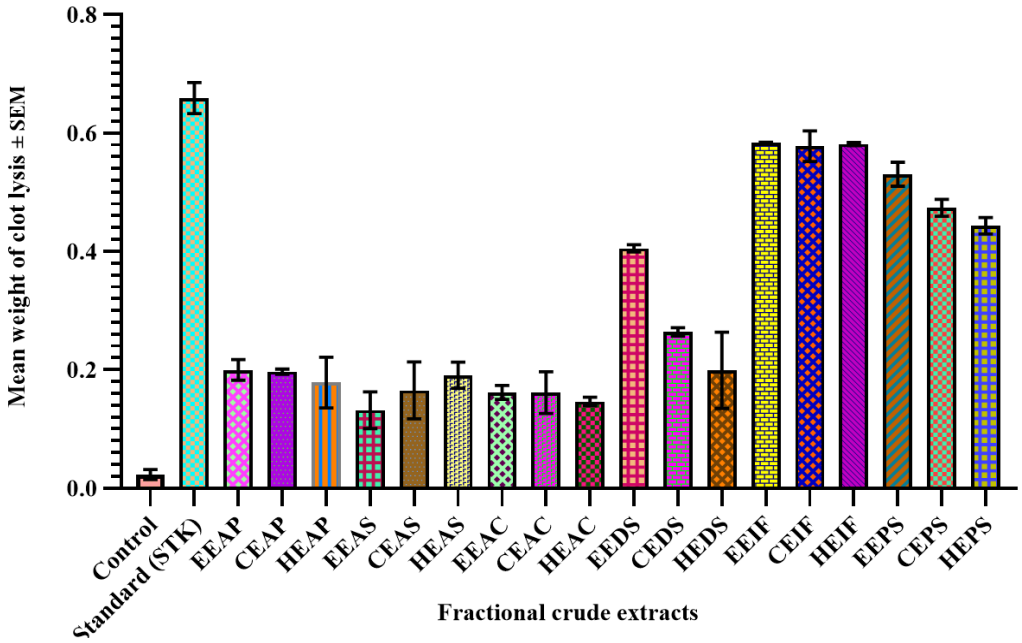

Fig. 2: The mean weights (g) of clot lysis activities of six medicinal plants were evaluated.

The CEIF (0.577±0.031) followed by HEIF (0.581±0.003) and EEIF (0.583±0.001) from *I. finlaysoniana* demonstrated more significant clot lysis activities compared to those from other plants. The p value *p<0.001*, compare with control (one way ANOVA followed by Tukey post hoc test). The present study reported that bioactive compounds from plant extracts were analysed, and results confirmed that the existence of alkaloids, glycosides, flavonoids, terpenoids, saponins, and vitamin C tannins provides biological activities. Extracts from plants (bioactive compounds) depend on the solvent mixture variations, ranging from low polarity to high polarity. Alkaloids have bioactive compounds producing analgesic, anti-inflammatory, and antibacterial activities, while terpenoids are well known for their antibacterial, anticancer, anti-inflammatory, analgesic and antiviral properties (Nassar et al. 2010). Glycosides are important for congestive heart failure and anticoagulants, anti-tumours, and influenza virus inhibition (Hashmi et al. 2021). All fractional (ethanol, chloroform and n-hexane) crude extracts of six selected plants confirmed that such types of bioactive compounds were identified through a rigorous qualitative testing experiment, involving specific methods. Among the tested medicinal plants, *P. sanguinea* and *A. paniculata* stood out with their remarkable ability to reduce heat-induced protein denaturation. Their effects surpassed those of acetyl salicylic acid and other plants, which exhibited effects ranging from mild to significant. The ethanol extracts of *P. sanguinea* and *A. paniculata* have shown potential in inhibiting protein denaturation and stabilizing cell membranes against lysis (Leelaprakash and Das 2011). Protein denaturation, a biological reaction during extended inflammatory responses, can lead to a loss of tissue function (Ikwegbue et al. 2018). The unique properties of the ethanol fraction of *P. sanguinea* and *A. paniculata*, which inhibit protein degradation and prevent cell membrane lysis, make them promising candidates for anti-inflammatory drug development. Numerous studies have been conducted to identify plants, natural food sources, and supplements that exhibit anti-thrombolytic effects, including antiplatelet and anticoagulant properties. Evidence suggests that the consumption of such foods may contribute to the prevention of coronary events and strokes (Joshipura et al. 1999, Liu et al. 2000, Bazzano et al. 2002). Platelets play a crucial role in atherothrombosis, a condition characterized by endothelial damage caused by reactive oxygen species. When activated, platelets form bonds with other platelets and leukocytes, contributing to the complex process of plaque development and progression. Plasmin, a natural fibrinolytic agent, works by degrading clots through the hydrolysis of fibrinogen and fibrin present within them. Streptokinase forms a 1:1 stoichiometric complex with plasminogen, which then converts additional plasminogen into plasmin (Banerjee et al. 2004). Numerous studies have shown that medicinal plants contain compounds such as tannins, alkaloids, and saponins, which may enhance their clot lysis activity (Ali et al. 2013, Hasanuzzaman et al. 2013). The present findings showed that the *I. finlaysoniana* and *P. sanguinea* reveal that these plants contain these types of phytochemicals, which contribute to their clot lysis activities.

Conclusion

The experimental data suggests that ethanol extracts from *P. sanguinea* leaves may possess therapeutic effectiveness, resulting in a 62.52% suppression of protein denaturation. The chloroform fraction of *I. finlaysoniana* had a clot lysis of 55.43%. The gathered findings suggest that *P. sanguinea* has considerable implications for anti-inflammatory effects, whereas *I. finlaysoniana* is associated with cardiovascular health. The findings can be used to develop herbal remedies for conditions such as inflammation and cardiovascular issues. It is crucial to perform more sophisticated investigations to isolate these chemicals and to determine which individual molecules are responsible for the reported effects.

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References

Ahsan MQ, MT Alam, MMU Chowdhury, MT Nasim and SMS Islam (2021). *In vitro* and *in vivo* evaluation of pharmacological activities of *Pouzolzia sanguinea.* Journal of Bio-Science 29(2): 31-42.

Ahsan MQ, Sikder MI, Rateep N, Nasim MT and Islam SMS (2025). Anti-inflammatory, analgesic & anxiolytic effects of crude extract and isolated bioactive fractional compounds from *Pouzolzia sanguinea*. MDPI Targets 3(19): 1-15.

Ali MS, Amin, MR, Kamal MI and Hossain MA (2013). *In vitro* antioxidant, cytotoxic, thrombolytic activities and phytochemical evaluation of methanol extract of the *A. philippense* L. leaves. Asian Pacific Journal of Tropical Biomedicine 3(6): 464-469.

Amin MN, Banik S, Ibrahim M, Moghal MR, Majumder MS, Siddika R, Alam MK, Jitu RM and Anonna SN (2015). A Study on *Ardisia solanacea* for evaluation of phytochemical and pharmacological properties. International Journal of Pharmaceutical and Phytopharmacological Research 7(1): 8-15.

Banerjee A, Chisti Y and Banerjee UC (2004). Streptokinase-a clinically useful thrombolytic agent research review paper. Biotechnology Advances 22(4): 287-307.

Bazzano LA, He J, Ogden LG, Loria CM, Vupputuri S, Myers L and Whelton PK (2002). Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first national health and nutrition examination survey epidemiologic follow-up study. The American Journal of Clinical Nutrition 76(1): 93-99.

Chowdhury Z, Alamgir ANM, Alauddin M, Islam MS, Chakma K, Hoque MR and Kabir MG (2008). Traditional knowledge related to medicinal and aromatic plants in tribal societies and the quantitative study of alkaloids in medicinal plants of the hill tracts in Bangladesh. Pharmacognosy Magazine 4(15): 137-144.

Faruque MO, Uddin SB, Barlow JW, Hu S, Dong S, Cai Q, Li X and Hu X (2018). Quantitative ethnobotany of medicinal plants used by indigenous communities in the Bandarban District of Bangladesh. Frontiers in Pharmacology 9(1): 1-12.

Ghani A (2005). Practical Pharmacognosy Textbook, 35rd Ed. Parash Publishers, Dhaka, pp. 12-8.

Habib T, Rahman A, Nair AB and Islam SMS (2023). Application of three compounds extracted from *Cynodon dactylon* against *Streptococcus mutans* biofilm formation to prevent oral diseases. Biomolecules 13(9): 1292, https://doi.org/10.3390/biom13091292.

Hasanuzzaman M, Ali MR, Hossain M, Kuri S and Islam MS (2013). Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of *Averrhoa bilimbi* (fruits). International Current Pharmaceutical Journal 2(4): 92-96.

Hashmi HF, Bibi S, Anwar M and Khan MR (2021). Qualitative and quantitative analysis of phytochemicals in *Lepidium pinnatifidum* Ledeb. Scholars International Journal of Traditional and Complementary Medicine 4(5): 67-75.

Ikwegbue PC, Masamba P, Oyinloye BE and Kappo AP (2018). Roles of heat shock proteins in apoptosis, oxidative stress, human inflammatory diseases, and cancer. Pharmaceuticals 11(2): 1-18.

Joshipura KJ, Ascherio A, Manson JE, Stampher MJ, Rimm EB and Speizer FE (1999). Fruit and vegetable intake in relation to risk of ischemic stroke. Journal of the American Medical Association 282(13): 1233-1239.

Kupchan SM, Britton RW, Ziegler MF and Sigel CW (1973). Ruceantin, a new potent antileukemic simaroubolide from *Brucea antidysenterica*. Journal of Organic Chemistry 38(1): 178-179.

Larayetan R, Ololade ZS, Ogunmola OO and Ladokun A (2017). Phytochemical constituents, antioxidant, cytotoxicity, antimicrobial, antitrypanosomal, and antimalarial potentials of the crude extracts of *Callistemon citrinus*. Evidence-Based Complementary and Alternative Medicine 5(1): 1-14.

Leelaprakash G and Dass MS (2011). *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. International Journal of Drug Development and Research 3(3):189-196.

Liu S, Manson JE, Lee IM, Cole SR, Hennekens CH, Willett WC and Buring JE (2000). Fruit and vegetable intake and risk of cardiovascular disease: the women's health study. The American Journal of Clinical Nutrition 72(4): 922-928.

Nassar ZD, Aisha AA and Majid AMS (2010). The pharmacological properties of terpenoids from *Sandoricum koetjape*. Webmedcentral 1(12): 1-11.