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Preliminary Phytochemical and Biological Investigations on Different Fractional Extracts of *Sarcochlamys pulcherrima* (Roxb.) Leaves

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Abstract

The current study aimed to investigate the phytochemicals & anti-inflammatory, antioxidant, thrombolytic and membrane stabilization properties of the *Sarcochlamys pulcherrima (Roxb.)* leaves by using various fractional extracts. The present study found alkaloids, tannins, flavonoids, reducing sugar, gums and amides in its hydroalcoholic extract; flavonoids and saponins in its chloroform extract of steroids and amides in its n-hexane extract. The maximal inhibition values were found for the hydroalcoholic and chloroform fractions in the antioxidant test at a dosage of 100 µg/ml. In addition, at 500 µg/ml concentration, all three extracts demonstrated substantial activity in the anti-inflammatory test accounted for 89.1%, 88.5% and 82.8% for hydroalcoholic, chloroform and n-hexane extracts respectively, compared to the 95.9% for standard (Diclofenac-Na) at the same concentration. In case of thrombolytic activity, the tested extracts showed very weak clot lysis compared to the reference standard, streptokinase. In the membrane stabilization activity test, hydroalcoholic, chloroform and n-hexane extracts were found to be 52.67%, 50.21%, and 15.52% at 1000 µg/ml, respectively. In comparison, the standard (Aspirin) showed 67.11% at 1000 µg/ml. Thus, overall findings suggested that the hydroalcoholic and chloroform extracts possess significant membrane-stabilizing activity at 1000 µg/ml.

Key words: *S. pulcherrima*, antioxidant, anti-inflammatory, thrombolytic, membrane *stabilizing.*

Introduction

The flowering plant family known as the Urticaceae is sometimes referred to as the nettle family. The genus *Urtica* gives rise to the family name. According to the database of the Royal Botanic Gardens, (Landén and Ståhle, 2016), the family has roughly 2,625 species, arranged into 53 genera (Chomicki and Renner, 2015). It is a little evergreen tree that may be found in floodplain secondary forests and tropical rainforests in Bhutan, Indonesia, Myanmar, Sikkim and Thailand. In the northeastern section of India, the plant is found growing wild in both plains and mountainous areas (Ibrahim *et al.*, 2014).

The usage of *Sarcochlamys pulcherrima* in conventional medical practices is widespread. This plant is traditionally used as food and medicine by several ethnic groups and communities in Bangladesh, Assam, Meghalaya and Nagaland, all of which are states of India (Ibrahim *et al.*, 2014). The

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material in this review is based on discussions with local residents of Assam's numerous communities as well as years' worth of research on the region's ethnobotanical claims, biological activities, and other topics. The scientific name for *S. pulcherrima* is dogal tree (English). In the Bandarban area of Bangladesh, the plant is referred to by the names Ma Cha Da (for the Marma tribe), Kan Leng (for the Murang tribe), Jung Gallya sak (for the Tanchangya tribe) and Jangaillya shak (for the Chakma tribe) (Mazumder *et al.*, 2015).

As part of our ongoing investigation into the therapeutic plants of Bangladesh, the current study aimed to evaluate the *in-vitro* antioxidant, anti-inflammatory, thrombolytic and membrane stabilization effects of crude extracts of *S. pulcherrima* leaf. This study was undertaken to determine the rationality of the plant's conventional uses.

Materials and Methods

Plant material collection: The leaves of *S. pulcherrima* were collected from Hathazari, Chattogram, Bangladesh and an identification specimen of the plant was kept at the herbarium of the Bangladesh Forest Research Institute in Chittagong, Bangladesh, where the experts made the taxonomic identification of this species.

Drying and grinding: After collection, the extraneous, undesired substances such as dust were removed from the leaves and then washed with running tap water. The plant was dried out in the shade at room temperature. They were then ground into a coarse powder in a grinder. The powder was stored in a sealed container at a cool, dark and dry area until extraction began.

Hot extraction by Soxhlet extractor: In a Soxhlet, 350 gm of powder was extracted with 1000 ml of 99.7% ethanol. The extracted solvent was collected, filtered and evaporated at below 50°C temperature. After solvent evaporation, a gummy concentration known as hot ethanolic crude extracts was produced (yield 8.27%).

Chemicals: All of the chemicals as well as reagents utilized in this study were from the German Merck Company and were of analytical grade.

Biological investigation: The solvent-solvent partitioning procedure developed by Kupchan and modified by Van Wagenen *et al.,* was utilized to put the ethanolic crude extract into the partitioning process. n-Hexane and then chloroform was used to separate the crude ethanol extract (28.946 g), which was first diluted in double-distilled water (DDW). Up to 150 ml of n-hexane and chloroform were used for fractionation; each time, 50 ml of solvent was added, and the mixture was vigorously shaken before being left to stand. Layers of solvent were divided and decanted. The remainder of the extract was utilized to create a hydro-alcoholic fraction of ethanol.

Phytochemical analysis: The presence of several phytoconstituents including alkaloids, glycosides, steroids, tannins, saponins, reducing sugar, amides, gums and flavonoids was examined in the leaf extracts of *S. pulcherrima*. All phytochemical tests were done as per the procedure given in the standard book (Gokhale, 2008). The various solvent fractions of *S. pulcherrima* were subjected to preliminary phytochemical screening using established techniques, (Ahamed *et al.*, 2021) to assure the presence of phytochemical substances.

DPPH method: The proposed approach, with a few minor modifications, (Brand Williams *et al.*, 1995) was used to determine the antioxidant capacity of the plant samples using the DPPH test (Islam *et al.*, 2019).

Anti-inflammatory activity: The anti-inflammatory properties of *S. pulcherrima* extractives was assessed by inhibition of egg albumin denaturation method (Ahamed *et al.,* 2021). In a nutshell, 0.2 ml of egg albumin and 2.8 ml of phosphate buffer were added to different concentrations of plant extract at 500, 250 and 125 µg/ml as well as in standard at the corresponding concentrations that were the same as those of the crude extract. A small quantity of 0.1 N HCl was used to correct pH of each of the aforementioned solutions. The samples were heated for five minutes

at 70°C after 15 minutes of incubation at 37°C. After cooling, UV spectrophotometer was used to measure the absorbance at 660 nm.

The percentage of protein inhibition was calculated using the formula below to determine the anti-inflammatory activity.

Inhibition percentage of protein denaturation

$$= \frac{A_{control} - A_{test}}{A_{control}} \times 100\%$$

Given, A= Absorbance for respective group.

Thrombolytic activity: Using the procedures outlined by (Chowdhury *et al.*, 2021) the capacity of extracts to prevent blood clots were assessed. In this test, streptokinase (SK) served as the standard.

Membrane stabilization assay: Anti-inflammatory medicines are used throughout the membrane stabilization process to maintain the membranes' stability. Lysosomal membrane lysis, which occurs during inflammation, releases enzymes responsible for a wide range of illnesses, including cancer and cardiovascular ailments. In this experiment, 16 fresh centrifuge tubes were used. The tubes were labelled as follows: three for standard, four for control and three for every different fractional extract of *S. pulcherrima.* 10% RBC suspension in 2 ml was added to all the tubes and then 2 ml of aspirin was added to the centrifuge tubes of the standard and control, and then 2 ml of the appropriate solvents (ethanol, chloroform and n-hexane) respectively were added to the tubes for the control. In contrast, 2 ml of various fractional extracts were combined in the test groups in the manner indicated. After that, 2 ml of a hypotonic solution was administered to each tube. All of the centrifuge tubes holding the reaction mixture underwent a 30-minute incubation at 37°C. The absorbance of the supernatant was determined using a UV-visible spectrophotometer at 560 nm following incubation (37°C for 30 min.) and centrifugation (10 min. at 3000 rpm). Each test sample's membrane stabilization activity was calculated as follows:

Inhibition percentage of hemolysis

$$= \frac{A_{control} - A_{test}}{A_{control}} \times 100\%$$

Here, A= Absorbance for respective group.

Results and Discussion

The medicinal plant is a plentiful source of advantageous bioactive ingredients that might be exploited as a springboard for creating new medications. Phytochemical screening, a qualitative inquiry, is an essential step in determining the bioactive components present in plant extracts. (Patel *et al.*, 2011). The primary goal of phytochemical research is to better understand the class of secondary metabolites that are present in plant extracts. The understanding of these chemical substances is very beneficial for the production of drugs. The n-hexane, chloroform and hydroalcoholic extracts of *S. pulcherrima* included large amounts of the phytoconstituents identified in our original phytochemical investigation, including alkaloids, glycosides, steroids, tannins, saponins, reducing sugars and flavonoids that are demonstrated in table 1.

Table 1. Phytochemical analysis of *S. pulcherrima* extracts in n-hexane, chloroform and hydroalcoholic fractions.

Phytochemical	Test sample		
	Hydroalcoholic extract	Chloroform extract	n-hexane extract
Alkaloids	-	+	-
Glycosides	-	-	-
Steroids	-	-	+
Tannins	-	+	-
Flavonoids	+	+	-
Saponins	+	-	-
Reducing sugar	+	+	-
Gums	-	+	-
Amides	+	+	+

'+' = present, '-'= absent.

With ascorbic acid acting as a reference, the DPPH scavenging assay was utilized to assess the quantitative antioxidant activity of the fractionated extracts of *S. pulcherrima.* The DPPH compound accepts a hydrogen atom from an antioxidant molecule as the basis for the test (Anjum *et al.*, 2022). According to figure 1, the antioxidant potential of various solvent fractions, which is comparable to that of conventional ascorbic acid, was significant and concentration-dependent. The fractionated extracts of *S. pulcherrima* demonstrated notable DPPH free radical scavenging activities with inhibitions of 77.20% for the hydroalcoholic fraction, 73.08% for the chloroform fraction and 36.76% for the n-hexane fraction against ascorbic acid (99.41% inhibition) at a maximum concentration of 100 µg/ml. For the soluble fractions of hydroalcoholic, n-hexane and chloroform, the half minimum inhibitory concentration (IC_{50}) was 2.26, 7.50, and 2.22 µg/ml, respectively, whereas, the value for ascorbic acid was 1.02 µg/ml.

By eliminating unwanted stimuli like infections, wrecked cells, or irritants from the living system, inflammation is regarded as a fundamental stage of the healing process (Landén *et al.*, 2016). In the current study, *in-vitro* anti-inflammatory efficacy of extracts was compared to the denaturation of egg albumin. At a concentration of 500 µg/ml, all three extracts demonstrated appreciable activity when compared to the reference medication (diclofenac-Na). For hydroalcoholic, chloroform and n-hexane extracts, they are 89.1%, 88.5% and 82.8%, respectively. The standard drug, diclofenac-Na, possessed 95.9% activity at the same concentration. At the concentration of 250 µg/ml a noteworthy activity was possessed by all three extracts. But the activity was remarkably dropped in the case of the dose of the three extracts at 125µg/ml. The results are shown in figure 1. The overall findings suggested evidence of significant anti-oxidant activity in hydroalcoholic and chloroform extracts.

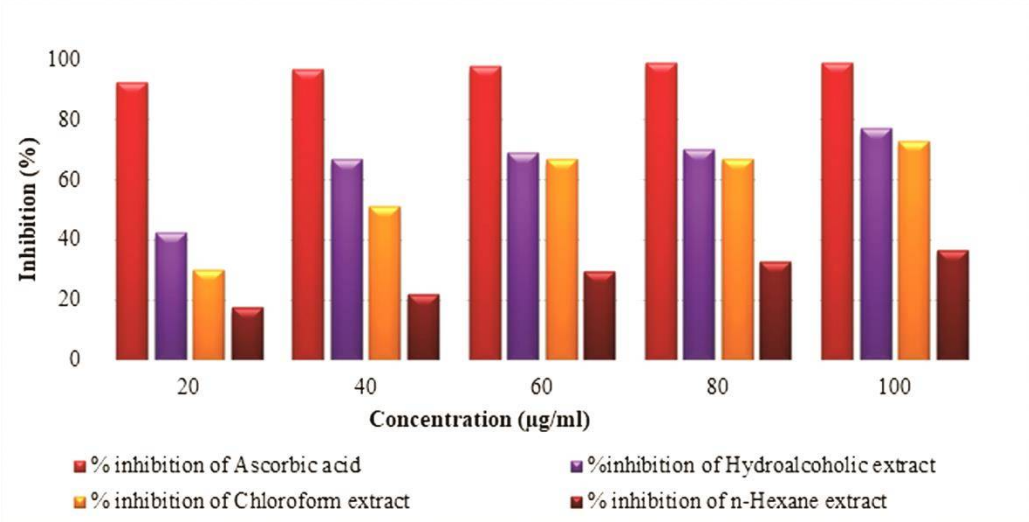

Figure 1**.** Antioxidant properties of n-hexane, chloroform and hydroalcoholic soluble fractions of *S. pulcherrima.*

In this study, the RBC membrane stabilization method was employed to further investigate *S. pulcherrima's* anti-inflammatory efficacy. Since human RBC membranes are comparable to the liposomal membrane, the prevention of RBC membrane breakdown has been used as a metric to evaluate plant extracts' ability to reduce inflammation. As is observed in figure 2A and 2B, the anti-inflammatory activity was concentration-dependent in both *in vitro* models.

The hydroalcoholic and chloroform extracts *S. pulcherrima* possess significant membrane stabilizing activity with 41.61% and 43.55% at 250 µg/ml; 48.39% and 46.41% at 500 µg/ml; and 52.67% and 50.21% at 1000 µg/ml. on the other hand the standard (Aspirin) showed 53.59% at 250 µg/ml, 66.44% at 500 µg/ml, and 67.11% at 1000 µg/ml (figure 2B)

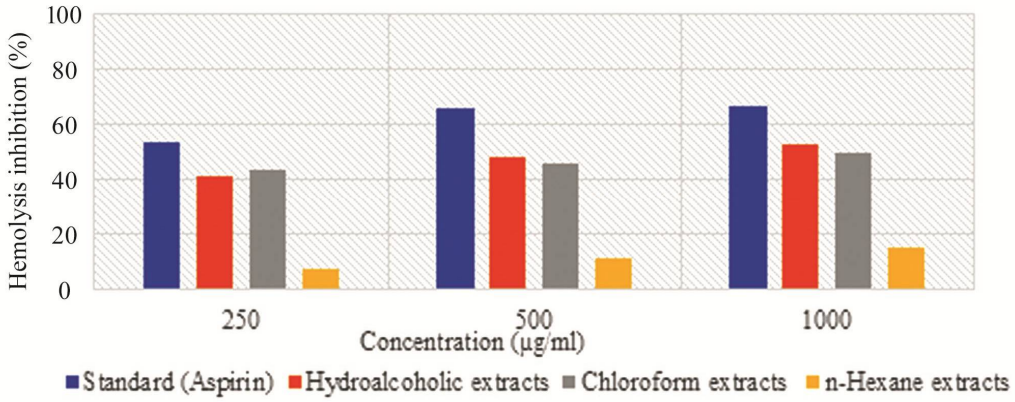

Figure 2A. Anti-inflammatory effect of n-hexane, chloroform and aqueous fractions of *S. pulcherrima* by inhibiting protein denaturation.

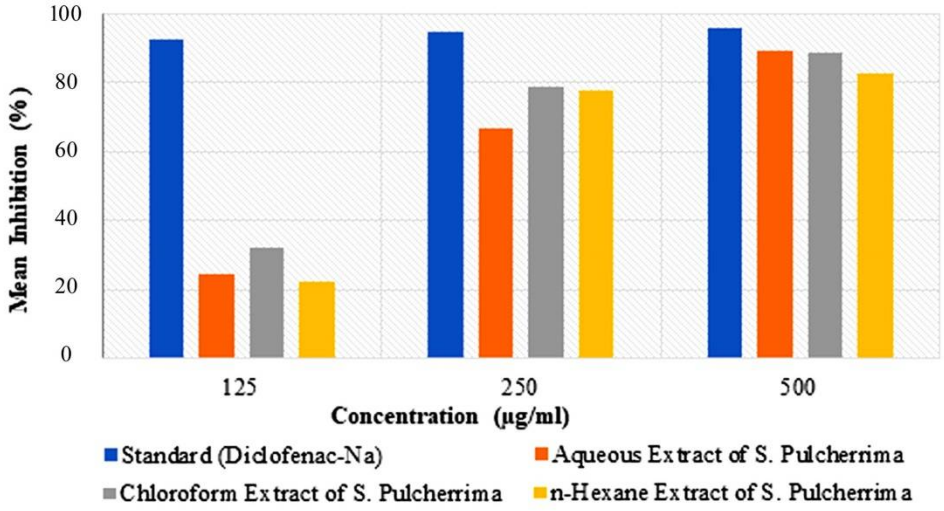

Figure 2B. The n-hexane, chloroform and aqueous fractions of *S. pulcherrima* exhibit anti-inflammatory efficacy by RBC membrane stabilization technique.

The clot lysis assay was used to assess the thrombolytic activity of the various fractional extracts of *S. pulcherrima* throughout the thrombolytic property screening process, with distilled water serving as the control and streptokinase serving as the standard test group. In the screening, the mean percentages of clot lysis for the hydroalcoholic, chloroform and n-hexane fractions were, 5.46%, 3.57% and 1.13% respectively, as opposed to the reference standard, streptokinase, which demonstrated 32.79% at 500 µg/ml. According to the aforementioned findings, all *S. pulcherrima* test samples exhibited a mild to low level of thrombolytic activity.

Medicinal herbs have many different ways of working that can be used to treat atherothrombosis. The numerous different qualities of plant extracts, such as antioxidant, anti-inflammatory, hypotensive, lipid-lowering, anti-thrombotic, etc., may indicate their capacity to avoid blood clots. Additionally, it has been determined that secondary plant metabolites including flavonoids, tannins, phenolics, etc., may function as thrombolytic agents (Kirichenko *et al.*, 2020).

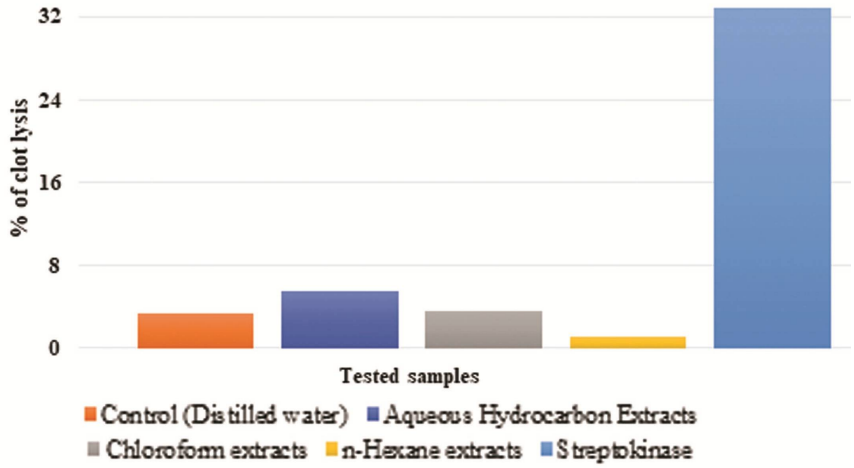

Figure 3. Thrombolytic activity of n-hexane, chloroform and aqueous soluble fractions of *S. pulcherrima*.

Conclusion

In our work, we emphasized the *in-vitro* antioxidant, anti-inflammatory, membrane stabilizing and thrombolytic properties of different solvent fractions of *S. pulcherrima.* Out of all the fractions, only the aqueous fraction showed evidence of possible antioxidant activity by scavenging the DPPH radical. Besides, the plant samples showed anti-inflammatory and membrane-stabilizing capabilities that were comparable to aspirin and standard diclofenac-Na, respectively. Thus, the research backs up the historical use of this plant in folk medicine. To comprehend the processes behind these bioactivities, more investigation is required.

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Conflict of interest

Authors have no conflict of interest.

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No funds have been received for this study.

Authors contribution

MAR and MI conceptualized, designed and supervised the study. Rest of all authors contributed equally to the study.

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