

Pharmacological Investigation on Ethanol Extract of *Scindapsus hederaceus* Miq.

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Abstract

The present study was conducted to evaluate the antipyretic, anti-inflammatory and membrane stabilizing, antioxidant, thrombolytic, anti-diarrheal activities of *Scindapsus hederaceus* belonging to the Araceae family. In antipyretic test, temperature reduced from 101.53°F to 99.86°F ($p < 0.05$), 99.20°F ($p < 0.05$) and 99.06°F ($p < 0.05$) in 1st, 2nd and 3rd hour, respectively and caused maximum reduction of temperature in 1st hour. In the hot plate method, the extract increased the reaction time of heat sensation significantly to 14.32 seconds. In *in vitro* anti-inflammatory test, the extract significantly inhibited protein denaturation by 85.17% at 500 µg/ml, 71.72% at 250 µg/ml and by 66.55% at 125 µg/ml. It also inhibited the hypotonic solution-induced haemolysis by 73.19%, 49.69% and 29.15% at same concentration in membrane stabilizing assay. In DPPH inhibition assay the extract showed maximum % inhibition of 65.5% at 100 µg/ml. In the assay of thrombolytic activity, the extract showed thrombolytic activity of 14.39%. Amylase inhibitory activity was found to be 22.38% at a concentration 100 µg/ml. In case of antidiarrheal investigation, the extract revealed total inhibition of defecation by 35.30%.

Key words: *Scindapsus hederaceus* Miq., anti-inflammatory, membrane stabilizing, thrombolytic, antidiarrheal, antipyretic.

Introduction

Science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations (LaValle *et al.*, 1999). A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. This definition of Medicinal plant has been formulated by World Health Organization (WHO, 2002). It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins, possess medicinal properties (Sofowara,

1982). WHO estimates that herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side-effects (Rao *et al.*, 2011). The use of natural substance, particularly plants, to control diseases is centuries-old practice that has led to the discovery of more than half of all "Modern" pharmaceuticals. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents (Mahesh, 2008). *S. hederaceus* a member of the family Araceae. It is a large climbing herb where the stem is slender, with clasping roots, fruit is a red in color of berry, seed is subreniform, compressed and thick, and the leaves are arranged

alternate, simple and entire, elliptical-lance-shaped (De Padua and Bunyaphatsara, 2003). The fruit is credited with stimulant, diaphoretic, aphrodisiac, carminative and anthelmintic properties, and is used to stop diarrhea and as an expectorant to treat asthma. It also has antiprotozoal activity and is applied externally to treat rheumatism.

Materials and Methods

Plant materials and extraction: Fresh plants were collected from the hill tracts area of Ramu, Cox'sbazar, Chittagong, in the month of February where taxonomical identification of this plant was made by the experts of Bangladesh Forest Research Institute (BFRI) Herbarium, Chittagong. After removing the extraneous, undesired substances from the plant material by hands, the soil was removed by sieving and the leaves were then subjected for shade dry at temperature not exceeding 50°C (Evans, 2002). After grinding, the powder was stored in airtight containers until extraction was commenced. For hot extraction, about 90 gm powder of the plant was subjected with 700 ml of ethanol (99%) in a Soxhlet apparatus (Bhaland Bhal, 1992).

Anti-inflammatory activity: The reaction mixture consisted of 3 ml of 5% egg albumin (from fresh hen's egg) solution prepared and 3 ml of varying concentrations of the test extract so that final concentrations become 125, 250 and 500 µg/ml, respectively. Similar volume of ethanol served as control. The pH (5.6±0.2) of the all reaction mixtures was adjusted by 0.1N HCl. Then the mixtures were incubated at 37±2 °C in a BOD incubator (Labline Technologies) for 15 minutes and then heated at 57 °C for 20 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Acetyl salicylic acid (Aspirin) at the same concentration range was used as reference drug and treated similarly for determination of absorbance (Sarafet al., 1999).

Membrane stabilizing activity: The membrane stabilizing activity of the extractives was evaluated by the inhibition of heat- and hypotonic solution induced haemolysis of human erythrocytes following the method developed by Omaleet al. (Omaleand Okafor, 2008).

DPPH free radical scavenging activity: Following the method developed by Brand-Williams (Berset et al., 1995), the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxy toluene (BHT) and ascorbic acid as reference standards.

Thrombolytic activity: The method developed by Prasad and Harbertson (Daginawalaet al., 2007) was used to determine the thrombolytic activity by using streptokinase (SK) as positive control. Aliquots (5 ml) of venous blood were drawn from healthy volunteers that were distributed in five different pre-weighed sterile alpin tubes (500 µL/tube) and incubated at 37°C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot was again weighed thereafter to determine the clot weight.

Antidiarrheal activity: Antidiarrheal activity was assessed by the castor oil-induced diarrhea in mice (Shoba and Thomas, 2001).

Antipyretic activity: This study was conducted by slightly modifying the method described by Adams (Nicholson et al., 1968). The experimental mice which showed at least an increase in rectal temperature to at least 34.27 °F, 18 hrs after Brewer's yeast injection. The crude methanol extract (BLME) exhibited a significant (p=0.05) lowering of mice's body temperature which was elevated by the administration of yeast. These effects were pronounced at the 2nd and 3rd hour post-treatment with extract. The antipyretic effects of the extract were comparable to that of the standard paracetamol.

Results and Discussion

The effect of ethanol extract of *S. hederaceus* on mice is presented in table 1. In this test, the extract at a dose of 500 mg/kg b.w significantly attenuated hyperthermia in mice up to 3 hours. Throughout the experiment, the extract reduced temperature from 101.53°F to 99.86°F, 99.20°F and 99.06°F in 1st, 2nd and 3rd hour respectively. The antipyretic activity of the extract was significant (p<0.05) compared to the control.

In the study of *in vitro* anti-inflammatory effect, *S. hederaceus* was evaluated against denaturation of egg albumin. The results are summarized in table 2. Acetyl salicylic acid was used as reference drug in the experiment at the concentration range (500-125 µg/ml) which exhibited concentration dependent inhibition of protein denaturation. The present experiment exhibited a concentration dependent inhibition of protein denaturation by 85.17%, 71.72% and 66.55% with *S. hederaceus* throughout the typical concentration range of 500 µg/ml, 250 µg/ml and 125 µg/ml.

In the study of membrane stabilization activity, the ethanol extract of *S. hederaceus* at concentration range of 500, 250 and 125 µg/ml protected significantly the erythrocyte membrane against lysis induced by hypotonic solution shown in table 3. Aspirin (500-125 µg/ml) also offered a significant (97.02%, 77.15% and 61.59%) protection of the RBC's against the damaging effect induced by hypotonic solution. At a concentration range of 500 - 125 µg/ml, the extract showed 73.19%, 49.67% and 29.15 % inhibition of hypotonic solution-induced haemolysis when compared with the standard.

Table 1. Antipyretic effect of ethanol extract of *S. hederaceus* on Swiss albino mice.

Groups	Oral dose	Rectal temperature in °F at different hours				
		-24 hr	0 hr	1 hr	2 hr	3 hr
Control (DDW)	10 mg/kg	98.36	101.50	101.20	101.25	101.40
Paracetamol	150 mg/kg	98.50	101.48	97.24	98.66	98.61
EESH	500 mg/kg	98.58	101.53	99.86	99.20	99.06

DDW= Double distilled water; *p < 0.05, EESH = Ethanol extract of *S. hederaceus*

Table 2. Spectrophotometric determination of anti-inflammatory activity of EESH.

Test groups	Mean inhibition of protein denaturation		
	500 µg/ml	250 µg/ml	125 µg/ml
Positive control (Aspirin)	93.12	89.65	79.31
EESH	85.17	71.72	66.55

EESH= Ethanol extract of *S. hederaceus*

Table 3. Spectrophotometric determination of membrane stabilization activity of EESH.

Test groups	Mean inhibition of haemolysis		
	500 µg/ml	250 µg/ml	125 µg/ml
Blank(Ethanol)	0	0	0
Positive control (Aspirin)	97.02	77.15	61.59
EESH	73.19	49.67	29.15

EESH= Ethanol extract of *S. hederaceus*

In DPPH scavenging assay the Ethanol extract showed maximum % inhibition of 65.5% at 100 µg/ml while the reference standard ascorbic acid showed % inhibition of 91.55% at the same concentration as shown in table 4. The DPPH radical scavenging activity was increased by increasing the concentration of the

sample extract. The extract exhibited considerable DPPH free radical scavenging activity as indicated by their IC₅₀ values which indicate the potency of scavenging activity. Standard ascorbic acid was found to have an IC₅₀ 52.18 µg/ml. Ethanol extract of *S. hederaceus* showed IC₅₀ 83.93 µg/ml.

Table 4. Radical scavenging activity and IC₅₀ of ethanol extract of *S. hederaceus* by DPPH.

Test groups	Concentration (µg/ml)	Absorbance (517 nm)	% Inhibition of DPPH	IC ₅₀ µg/ml
Control (Ethanol)	--	0.368	0	
Ascorbic Acid (Standard)	20	0.276	25.00	52.18 µg/ml
	40	0.197	46.46	
	60	0.132	64.23	
	80	0.097	73.68	
	100	0.009	91.55	
EESH	20	0.338	08.15	83.93 µg/ml
	40	0.288	21.74	
	60	0.245	33.43	
	80	0.208	43.48	
	100	0.127	65.50	

EESH= Ethanol extract of *S. hederaceus*

Results of thrombolytic activity of the plant extracts, control (double distilled water), standard drug (streptokinase), were shown in table 5. In this study, the extractives of *S. hederaceus* showed mild thrombolytic activity in comparison to the standard streptokinase showing 81.54% clot lysis. The extract showed thrombolytic activity 14.39% (of clot lysis). At the same time ethanol was treated as negative control which exhibited negligible 8.47% lysis of clot.

Table 5. Thrombolytic activity of the test groups.

Thrombolytic activity of Test Groups	Total lysis
Negative control (ethanol)	8.47± 0.6
Positive control Streptokinase (100 µL)	81.57 ± 3.705
Ethanol extracts of <i>S. hederaceus</i> (5 µg/µL)	14.39 ± 0.80

Table 6. Inhibition of defecation by ethanol extract of *S. hederaceus*.

Test groups	% of inhibition defecation
Negative control	0.00
Positive control (Loperamide)	58.87±0.33
EESH	35.30±0.88

EESH= Ethanol extract of *S. hederaceus*

In the castor oil induced diarrheal mice, the ethanol extract of *S. hederaceus* at the dose of 500mg/kg showed total 35.30% inhibition of defecation compared to standard, loperamide (3 mg/kg) with total 58.87% inhibition of defecation. (Table 6)

Conclusion

Antipyretic activity of *S. hederaceus* revealed the significant fever attenuating potential of the plant with significant ($p < 0.05$) analgesic potential in the extract. The extract of *S. hederaceus* significantly protected the erythrocyte membrane against lysis induced by hypotonic solution and having good anti-inflammatory activity comparable with that of the standard acetyl salicylic acid. It showed significant antioxidant activities in DPPH inhibition assay with mild thrombolytic and moderate anti-diarrheal effect. So, *S. hederaceus* showed statistically significant activities in those tests conducted. The results verify some of the traditional uses of the plant. For instance, further investigation may be carried out to isolate and characterize the active principle of the plant and to elucidate the exact mechanisms of action.

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