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RESEARCH ARTICLE

Pharmacological and Phytochemical Screenings of Ethanol Extract of *Sterculia villosa* Roxb.

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ABSTRACT

The antimicrobial, anti-inflammatory, membrane stabilization and anti-atherothrombosis activities of crude ethanol extract of leaves of *Sterculia villosa* Roxb. has been investigated. In antimicrobial assay by disc diffusion method, the extract showed mild to moderate antimicrobial activity with zone of inhibition ranging from 11-14 mm and 7-13 mm for test bacteria and fungi, respectively where the growth of *Salmonella paratyphi* and *Pityrosporium ovale* was strongly inhibited. In the *in-vitro* anti-atherothrombosis test, the crude extract exhibited 19.62% clot lysis as compared to the standard, streptokinase (81.53%). Moreover, the extract inhibited protein denaturation and haemolysis by 48% and 69.49% in the *in-vitro* anti-inflammatory and membrane stabilization test, respectively. Preliminary phytochemical screenings with the crude extract revealed the presence of alkaloids, glycosides, tannins, flavonoids, reducing sugars and gums.

KEY WORDS: *Sterculia villosa*, Antimicrobial, Anti-inflammatory, Membrane stabilization, Anti-atherothrombosis.

INTRODUCTION:

Sterculia villosa Roxb. (Bengali: Udal) is one of the fast-growing plants; available in the forests of Chittagong and Chittagong Hill Tracts, Cox's Bazar, Gazipur, Tangail, Comilla and Habiganj. It is a medium-sized tree (about 15–18 m tall) with grey bark about 2.50–2.65 cm thick[1]. The plant possesses diuretic, cooling and aphrodisiac properties. Sherbet, prepared from the petiole along with water and sugar is given in urinary problems and rheumatism. Leaves are used for treatment of impotency in Habiganj. The bark and the petiole are used as a remedy in seminal weakness[2]. White exudate of the tree is used for throat infection. Root infusion is taken as food adjunct while the whole plant extract is useful for skin diseases[3].

As part of our ongoing research with medicinal plant of Bangladesh,[4,5] the present study has been undertaken to evaluate the antimicrobial, anti-inflammatory, membrane stabilization and anti-atherothrombosis activities of the species to find out evidence for its folk uses and to introduce it as a source of new drug candidate.

MATERIALS AND METHODS:

COLLECTION AND EXTRACTION:

The leaves of *S. villosa* were collected from the "Botanical Garden & Eco Park" Sitakundo, Chittagong, Bangladesh and were identified by Mr. Md. Mohiuddin, Director, Forest Research Institute, Chittagong, Bangladesh, where a voucher specimen has been maintained. After collection, the samples were sun dried for 7 d followed by oven drying for 24 h at 50 ºC to facilitate proper grinding. Then about 130 g of powdered leaf was extracted with ethanol (99.8%) in a Soxhlet apparatus (Quickfit, England) for 10 h and the extract thus obtained was concentrated with a rotary evaporator (Heidolph, 560-91110-00-0, Germany) at reduced temperature and pressure.

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PRELIMINARY PHYTOCHEMICAL SCREENINGS:

For preliminary phytochemical screenings, the crude extract was subjected to various tests (Table-1) for determination of chemical nature (secondary metabolites) of the extract.

ANTIMICROBIAL SCREENING:

The antibacterial and antifungal activities of the crude extract were evaluated by the disc diffusion method[6] against test organisms (Table-2) using ciprofloxacin (CIPROCIN 250 mg/Tab., Square Pharmaceuticals Ltd., Bangladesh) as standard. The organisms were obtained as pure culture from the Faculty of Microbiology, University of Chittagong, Bangladesh. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition (mm). The experiments were carried out in triplicate and the results have been shown as mean ± SEM (Standard Error of Mean).

MINIMUM INHIBITORY CONCENTRATION:

The minimum inhibitory concentration (MIC) of the crude extract was determined by the micro-dilution technique[7] in broth medium (Hi Media Laboratories, India), containing graded concentration of the plant extract inoculated with the test organisms (Table-2).

ANTI-INFLAMMATORY ACTIVITY:

To conduct the experiment, 3 clean centrifuge tubes were taken for standard (positive control), negative control and crude ethanol extract. 1.0 ml of 5% egg albumin solution was added to the tubes. Then 1.0 ml of ethanol was added to the control tubes. 1.0 ml acetyl salicylic acid (0.1%) was added as positive control group. On the other hand for test group 1 ml ethanol extract (500 µg/ml) was mixed to the "test" marked tube. The pH (5.6 ± 0.2) of the all reaction mixtures was adjusted with 1N HCl and heated at 57 °C for 20 min. After cooling and filtering through Whatman no. 1 filter paper, the absorbance was measured spectrophotometrically at 660 nm. The test was repeated for three times.

MEMBRANE STABILIZING ACTIVITY:

The membrane stabilizing activity was assessed by using hypotonic solution induced hemolysis of human erythrocyte[8]. For this study, 3 clean centrifuge tubes were taken for standard, positive control and crude extract and marked accordingly. About 1.0 ml of 10% RBCs suspension was added to all tubes and 1.0 ml ethanol and 1.0 ml acetyl salicylic acid were added to the negative control and positive control marked tube, respectively. On the other hand, 1.0 ml of (500 µg/ml) crude extract was mixed to the test group. Then all the tubes were treated with 1.0 ml of hypotonic solution. The pH (7.4 ± 0.2) of the reaction mixtures was adjusted by phosphate buffer. All centrifuge tubes containing reaction mixtures were incubated at 56 °C for 30 min in a water bath. The tubes were cooled under running tap water and then centrifuged at 2500 rpm for 5 min. The absorbances of the supernatants were measured at 556 nm with a UV-visible spectrophotometer. The test was repeated for three times.

ANTI-ATHEROTHROMBOSIS ACTIVITY:

The thombolytic activity of the crude extract was evaluated by previously described method[9] using streptokinase as standard. For this study, 4 ml venous blood was drawn from healthy volunteers and distributed in three (for extract, reference standard and for negative control) pre-weighed sterile microcentrifuge tubes (0.5 ml/tube) and incubated at 37 °C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube was weighed again to determine the weight of clot (clot weight = weight of clot containing tube – weight of tube alone). Then, 100 µl of ethanol extract at a dose of 5 µg/µl, 100 µl of streptokinase and 100 µl of ethanol were separately added to the pre-marked tubes containing the clot. The tubes were then incubated at 37 °C for 90 min and observed for clot lysis. Afterwards, the fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated for three times in different days with fresh blood samples collected from 10 healthy volunteers (male and female) having no history of contraceptives and anticoagulants.

STATISTICAL ANALYSIS:

The primary data obtained from the experiments were manipulated as the source of responses. As indicated before, three samples were prepared for each of the bioassays and data were expressed as mean ± SEM (standard error of mean). Statistical analysis was performed by student's t-test (n=3). Differences were considered statistically significant when $p < 0.5$.

RESULTS AND DISCUSSION:

PHYTOCHEMICAL SCREENING:

In preliminary phytochemical screenings, the crude ethanol extract demonstrated the presence of various compounds i.e., alkaloids, glycosides, tannins, flavonoids, reducing sugars and gums (Table-1).

Table-1: Presumption for the phytoconstituents of the crude extract of *S. villosa*

Examination	Test performed	Result
Alkaloids	Meyer's test	+++
	Dragendorff's test	+++
	Wagner's test	++
	Hager's test	--
	Tannic acid test	++
Glycosides	Salkowski test	+++
	Libermann-Burchared test	+++
Steroids	Salkowski test	--
	Libermann-Burchared test	--
Tannins	Ferric chlorides test	++
	Potassium dichromate test	++
Flavonoids	Conc. HCl and alcoholic test	++
Saponins	Shake test (aq. solution)	--
Reducing sugar	Fehling's test	+++
	Benedict's test	+++
Gums	Molisch's test	++

(+) = present; (-) absent

PHARMACOLOGICAL STUDIES:

In the anti-bacterial activity test, the zone of inhibition was found within the range of 11 to 14 mm. The extract exhibited highest activity against *S. paratyphi* with zone of inhibition of 14 mm and MIC value of 125.0 µg/ml. It also showed moderate activity against *B. megaterium*, *S. aureus*, *E. coli*, *V. cholerae, S. typhi*, *S. dysenteriae*, *B. cereus*, *B. subtilis* and *Sh. sonnei.* During the anti-fungal screening, the highest zone of inhibition 13 mm was obtained against *P. ovale* (Table-2).

In the present study for in-vitro anti-inflammatory test, the crude extracts at the dose of 500 µg/ml showed 48% inhibition of protein denaturation whereas standard acetyl salicylic acid (ASA) by 52.35% (Table-3). The ability of ethanol extract was found to be significant in inhibiting heat induced protein denaturation.

The extract at 500 µg/ml inhibited the heat induced haemolysis of RBCs by 69.49% whereas standard aspirin showed 89.83% inhibition of haemolysis (Table-4). The stabilization activity for crude ethanol extract was found to be moderate. Although the precise mechanism of this membrane stabilization is yet to be elucidated, it is thought that the plant may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation.

In the anti-atherothrombosis activity test, streptokinase (a positive control) showed 81.53% clot lysis. On the other hand, clots when treated with 100 µl ethanol (negative control) showed only negligible lysis (2.49%). In the same time, treatment of clots with 100 µl of the extract, 19.62% clot lysis was obtained. Statistical representation of the effective clot lysis percentage has been shown in table-5.

Table-2: Antimicrobial activity of ethanol extract of *S. villosa*

Test organisms	Diameter of zone of inhibition (mm)		Minimum inhibitory concentrations (µg/ml)
	Ethanol extract (500 µg/disc)	Standard (30 µg/disc)	
Gram positive bacteria		**Ciprofloxacin**	
Bacillus cereus	12±1.00[a]	12.8±1.04	250
Bacillus megateriuum	13±1.00[d]	14.2±0.76	250
Bacillus subtilis	12±1.00[d]	14.8±1.04	250
Staphylococcus aureus	13±1.00[d]	12.3±0.58	125
Gram negative bacteria			
Escherichia coli	13±1.00[e]	14.7±0.58	125
Pseudomonas aeruginosa	nd	15.3±1.04	nd
Salmonella typhi	13±1.00[c]	15.5±0.50	250
Salmonella paratyphi	14±1.73[d]	12.5±0.50	125
Shigella dysenteriae	12±1.43[c]	12.5±1.50	250
Shigella sonnei	12±0.50[a]	13.8±0.29	250
Vibrio cholerae	13±1.73[d]	13.8±0.29	250
Fungi			
Aspergillus niger	7±1.15[a]	13.7±0.76	nd
Blastomyces dermatitidis	10±1.43[d]	11.7±0.76	125
Candida albicans	11±1.32[d]	11.5±1.50	250
Cryptococcus neoformans	nd	12.7±1.26	nd
Microsporum sp.	10±1.26[d]	11.5±1.32	nd
Pityrosporum ovale	13±1.50[d]	13.0±0.50	nd
Trichophyton sp.	11±1.73[d]	14.5±0.50	125

[a]p<0.01, [b]p<0.02, [c]p<0.05, [d]p<0.10, [e]p<0.50; The diameter of zone of inhibition is expressed as mean±SEM (n=3); SEM: standard error of mean; Zone of inhibition under 8 mm was considered as inactive and were discarded. nd: not detected

Table-3: *In-vitro* anti-inflammatory activity of test sample and controls

Test groups	Total inhibition of protein denaturation
Control	-
Positive control (ASA 0.1%)	52±0.0007[b]
EESV (500 µg/ml)	48±0.002[a]
[a]p<0.02, [b]p<0.001; Total inhibition of protein denaturation = % MIPD ±SEM; EESV: Ethanol extract of *S. villosa*	

Table-4: *In-vitro* membrane stabilization activity of test sample and controls

Test groups	Total inhibition of haemolysis
Control	-
Positive control (ASA 0.1%)	89.83±0.002[a]
EESV (500 µg/ml)	69.49±0.003[b]
[a]p<0.01, [b]p<0.02; Total inhibition of haemolysis = %IMHLs±SEM	

Table-5: Anti-atherothrombosis activity of test sample and controls

Treatment groups	Clot lysis (%)
Ethanol (Negative control)	2.49±0.39
Streptokinase (Positive control)	81.53±3.70[b]
EESV	19.62±1.04[a]
[a]p<0.01, [b]p<0.02; Values are expressed as mean ± SEM	

CONCLUSION:

The present work was conducted to investigate the antimicrobial, anti-inflammatory, membrane stabilization and anti-atherothrombosis activities of ethanol extract of *S. villosa* as well as to determine the chemical profiles of the extract. Preliminary phytochemical screenings demonstrated the presence of alkaloids, glycosides, tannins, flavonoids, reducing sugars and gums. This plant showed moderate antimicrobial activity. The ability of ethanol extract to inhibit thermal and hypotonic solution induced protein denaturation was found to be mild and provides evidence for poor membrane stabilization as well as anti-inflammatory effects. So, the results obtained from this study indicate that this plant species could be useful in the search for new natural bioactive compounds.

REFERENCES

1. S.R. Ghosh, P.P. Baruah. Regional Research Laboratory, Council of Scientific & Industrial Research, Jorhat-785006, Assam, India.
2. http://www.mpbd.info/plants/sterculia-villosa.php.
3. R.M. Kunwar**,** K.P Shrestha, R.W Bussmann. Traditional herbal medicine in Far-west Nepal: a pharmacological appraisal. J. Ethnobiol. Ethnomed., 2010, 6: 35.
4. M.R. Kuddus, F. Aktar, M.K. Miah, M.A. Baki and M.A. Rashid. Polyphenols content*,* cytotoxic, membrane stabilizing and thrombolytic activities of *Sarcolobus globosus*: A medicinal plant from Sundarban Forest. Bol. Latinoam. Caribe Plant Med. Aromat., 2011, 10: 363-68.
5. M.R. Kuddus, F. Rumi, M. A. Kaisar¸ M.S. Rahman, C.M. Hasan, M. A. Hassan and M.A. Rashid. Secondary metabolites from *Melocanna Baccifera* (Roxb.). Asian J. Chem., 2011, 23: 85-8.
6. A.W. Bauer, W.M.M. Kibry, J.C. Sheries, M. Turek. Antibiotic succeptibility testing by a standard single disc method. Am. J. Sci., 1951, pp.1, 103, 195.
7. R. Reiner. Detection of antibiotic activity. In antibiotic an introduction. Roche Scientific Service, Switzerland. 1982, pp. 1, 21-5.
8. U.A. Shinde, A.S. Phadke, A.M. Nari, A.A. Mungantiwar, V.J. Dikshit, M.N. Saraf. Membrane stabilization activity-a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia., 1999, 70: 251-57.
9. 9. S. Prasad, R.S. Kashyap, J.Y. Deopujari, H.J. Purohit, G.M. Taori, H.F. Daginawala. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thromb. J., 2006, 4**:** 14.