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Research Article

INVESTIGATION OF ANALGESIC AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *STREBLUS ASPER* LOUR. (MORACEAE) LEAF AND BARK

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ABSTRACT

The ethanol extract of the leaf and bark of *Streblus asper* Lour. (Moraceae) was investigated for its possible analgesic effects in animal models and its antioxidant activities. It exhibited statistically significant writhing inhibition in acetic acid induced writhing inhibition in mice. The crude extract of leaf of 500 mg/kg & 250 mg/kg body weight produced 65.46% & 27.79% inhibition of writhing. On the other hand bark of 500mg/kg & 250mg/kg body weight produced 58.70 % & 20.26% inhibition of writhing respectively; while the standard drug Diclofenac inhibition was found to 64.94 % at a dose of 25 mg/kg body weight. The antioxidant property of ethanolic extract of leaf & bark of *Streblus asper* Lour. was assessed by DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity. In DPPH scavenging assay the IC_{50} value of leaf & bark was found to be 1 µg/ml & 10 µg/ml which were comparable to the standard ascorbic acid.

Key words: *Streblus asper*, analgesic, antioxidant, DPPH

INTRODUCTION

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal plants". Medicinal plants may be defined as a group of plants that possess some special properties or virtues that qualify them as article of drugs and therapeutic agent and are used for medicinal purposes. According to WHO consultative group on medicinal plants, "A medicinal plant is any plant which, in one or more of its organs, contain substances that can be used for therapeutic purposes or which, is a precursor for synthesis of useful drugs. In our opinion, we should continue recognizing all those plants as medicinal which have been traditionally used over the years and are still being used for therapeutic purposes, some with spectacular reputation, until their efficacy is proved otherwise by scientific analysis and clinical evaluation. The current list of medicinal plants growing around the world includes more than a thousand items.

Streblus asper Lour. (Moraceae) is a small tree which is indigenous to tropical countries such as India, Sri Lanka, Malaysia, the Philippines and Thailand. It is known by various names, e.g. Bar-inka, Berrikka, Rudi, Sheora, Koi, Siamese rough bush and Tooth brush tree[1]. In India it is known by its several vernacular names, the most commonly used ones being Shakhotaka (Sanskrit), Siora (Hindi), Sheora (Bengali) and Piray (Tamil)[2]. It is used traditionally in leprosy, piles, diarrhea, dysentery, elephantiasis[3] and cancer[4]. It is a rigid shrub or gnarled tree; branchlets tomentose or pubescent. Leaves are 2–4 inch, rigid, elliptic, rhomboid, ovate or obovate, irregularly toothed; petiole 1/12 inch. Male heads globose, solitary or 2-nate, sometimes androgynous; peduncle short scabrid, flowers minute. Female flowers longer peduncled. Fruit pisiform; perianth yellow. It is found in the drier parts of India, from Rohilkund, eastward and southwards to Travancore, Penang and the Andaman Islands[5]. The pharmacognostical studies of its stem bark as well as its root bark have been carried out[6,7]. It finds place in the Ayurvedic Pharmacopoeia of India[8] and has also been described in some monographs[9]. *Streblus asper* Lour. is a well known medicinal plant which is also used in Ayurveda[2,10–14]. Its use in the Indian traditional folk medicine is also well documented.

MATERIALS AND METHODS

Plant material collection and extraction

For this present investigation, *Streblus asper* Lour. (Moraceae) was collected from Khulna region, Bangladesh in July, 2005 at morning time and identified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession No: 31116) and a voucher specimen also deposited there. The collected plant parts (leaves & barks) were separated from undesirable materials or plants or plant parts. They were shed-dried for four weeks. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 150 gm of powdered material (leaf) & 120 gm of powdered material (bark) was taken in two different clean, flat-bottomed glass containers and soaked in 800 ml & 800 ml of 80% ethanol in each container respectively. The containers with its contents were sealed and kept for a period of 12 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (ethanol extract) obtained was evaporated under ceiling fan until dried. It rendered greenish color powder 8.67gm (% yield) for leaf & green color concentrated paste of 5.5 gm (% yield) for bark. The concentrated paste extract of bark & dried powder of leaf was designated as crude extract or ethanolic extract.

Drugs and Chemicals

Diclofenac Sodium (Orion Pharma Ltd. Bangladesh), Chloroform, Methanol, n-Hexane, Acetone, Distilled water, DPPH (0.02 % w/v) solution in ethanol, Ascorbic Acid.

Animals

Young Swiss-albino mice of either sex, weighing 20-25 gm, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 - 65%, room temperature 25.0 ± 2.0°C and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals.

Pharmacological Studies

Analgesic activity

Analgesic activity of the crude extract of *Streblus asper* Lour. (Moraceae) was tested using the model of acetic acid-induced writhing in mice[15,16]. The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug Diclofenac sodium at dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with the extracts at dose of 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 minutes prior to acetic acid (0.7%) injection. Then after an interval of 15 minutes, the number of writhes (squirms) was counted for 5 min.

Antioxidant assay (Qualitative)

This test was performed to see the presence of antioxidant groups in the plant extract. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) is a stable free radical which is neutralized by the antioxidants. DPPH forms deep pink color when it is dissolved in ethanol. When it is sprayed on the chromatogram of the extract, it forms pale yellow or yellow color which indicates the presence of antioxidants in the sample.

For Polar solvent system: A little amount of ethanolic extract of plant (leaf & bark) were dissolved in ethanol and diluted suitably and was applied on the TLC plate by spotter (Capillary tube). TLC plate was then kept in a jar containing a solvent system of chloroform, methanol and water (40:10:1). A filter paper was kept in the jar and closed tightly. After developing the chromatogram the plate was air-dried & 0.02 % DPPH solution of ethanol was sprayed on it by a spray gun. Medium prominent yellow color was formed for leaf extract & yellow color was absent for bark extract on the chromatogram

For Medium polar solvent system: A little amount of ethanolic extract of plant (leaf & bark) was dissolved in ethanol and diluted suitably and was applied on the TLC plate by spotter (Capillary tube). TLC plate was then kept in a jar containing a solvent system of chloroform and methanol (5:1). A filter paper was kept in the jar and closed tightly. After developing the chromatogram the plate was air-dried & 0.02 % DPPH solution of ethanol was sprayed on it by a spray gun. Prominent yellow color was formed for leaf extract & Medium prominent yellow color was formed for bark extract on the chromatogram.

For Medium Non polar solvent system: A little amount of ethanolic extract of plant (leaf & bark) were dissolved in ethanol and diluted suitably and was applied on the TLC plate by spotter (Capillary tube). TLC plate was then kept in a jar containing a solvent system of n- Hexane and Acetone (3:1). A filter paper was kept in the jar and closed tightly. After developing the chromatogram the plate was air-dried & 0.02 % DPPH solution of ethanol was sprayed on it by a spray gun. Less prominent yellow color was formed for leaf extract & bark extract on the chromatogram.

Method evaluation: At first chromatogram was developed in the solvent system of chloroform, methanol and water (40:10:1) When DPPH was sprayed on it, medium prominent yellow color was formed for leaf extract & yellow color was absent for bark extract on the chromatogram then chromatogram was developed in the solvent system of chloroform and methanol (5:1). When DPPH was sprayed on it, prominent yellow color was formed for leaf extract & Medium prominent yellow color was formed for bark extract on the chromatogram. After that chromatogram was developed in the solvent system of n- hexane and acetone (3:1). When DPPH was sprayed on it, less prominent yellow color was formed both for leaf extract & bark extract on the chromatogram.

Antioxidant assay (Quantitative)

The anti-oxidant potential of the ethanolic extract was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquots of the different concentrations (1-500 μg/ml) of the extract of 5 ml were added to 5 ml of a 0.004% EtOH solution of DPPH. Absorbance at 517 nm was determined after 30 min, and IC_{50} (Inhibitory conc. 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The formula used for % inhibition ratio is-

$$\% \text{ inhibition} = (\text{Blank OD} - \text{Sample OD}) / \text{Blank OD} \times 100$$

At first 6 test tubes were taken to make aliquots of 6 conc. (1, 5, 10, 50, 100 and 500 μg/ml). Plant extract and ascorbic acid were weighed 3 times and dissolved in ethanol to make the required concentrations by dilution technique. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously, stirrer was used. After making the desired concentrations, 5 ml of 0.004% DPPH solution was applied on each test tube by pipette. The room temperature was recorded and kept the test tubes for 30 minutes in light to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank. After 30 minutes, absorbance of each test tube was determined by UV spectrophotometer. Then % inhibition was plotted against log concentration and from the graph IC_{50} was calculated. The experiment was performed in triplicate and average absorbance was noted for each concentrations. Ascorbic acid was used as positive control.

Table 1: Result of the analgesic effect of ethanolic extract of *Streblus asper* Lour. leaf

Animal group/ Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 in water, p.o.	77±4.39 (100)	-
Positive control Diclofenac sodium 25 mg/kg, p.o.	27±2.16* (35.05)	64.94
Test group - 1 Ethanolic extract 250 mg/kg, p.o.	55.6±2.63** (72.02)	27.79
Test group - 2 Ethanolic extract 500 mg/kg, p.o	26.6±0.98** (34.54)	65.46

Values are expressed as Mean S.E.M (n=5), *P<0.001, **P<0.01, % = Percentage, p.o. = per oral.

Table 2: Result of the analgesic effect of ethanolic extract of *Streblus asper* Lour. bark

Animal group/ Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 in water, p.o.	77±4.39 (100)	-
Positive control Diclofenac sodium 25 mg/kg, p.o.	27±2.16* (35.05)	64.94
Test group - 1 Ethanolic extract 250 mg/kg, p.o.	31.8±2.29** (41.29)	20.26
Test group - 2 Ethanolic extract 500 mg/kg, p.o	61.4±2.33*** (79.74)	58.70

Values are expressed as Mean S.E.M (n=5), *P<0.001, **P<0.01, ***P<0.02, % = Percentage, p.o. = per oral.

Table 3: Result of the DPPH scavenging assay of ascorbic acid

	Blank OD	Concentration of Ascorbic acid					
		1 µg/ml	5 µg/ml	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml
Absorbtion-1	0.537	0.248	0.051	0.021	0.017	0.015	0.009
Absorption-2	0.537	0.242	0.040	0.027	0.017	0.015	0.014
Absorption-3	0.524	0.248	0.040	0.021	0.018	0.011	0.017
Average Absorption	0.532	0.246	0.043	0.023	0.017	0.013	0.013
% Inhibition		53.76	91.92	95.68	96.80	97.56	97.56
log concentration		0	0.69	1	1.69	2	2.69

Table 4: Result of the DPPH scavenging assay of ethanolic extract of S*treblus asper* Lour. leaf

	Blank OD	Concentration of *Streblus asper* Lour (Leaf)					
		1 µg/ml	5 µg/ml	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml
Absorbtion-1	0.501	0.254	0.247	0.246	0.198	0.134	0.096
Absorption-2	0.517	0.256	0.247	0.243	0.189	0.133	0.104
Absorption-3	0.525	0.261	0.248	0.247	0.194	0.137	0.181
Average Absorption	0.514	0.257	0.247	0.245	0.194	0.135	0.127
% Inhibition		50.00	51.94	52.33	62.26	73.74	75.29
log concentration		0	0.69	1	1.69	2	2.69

Table 5: Result of the DPPH scavenging assay of ethanolic extract of S*treblus asper* Lour. bark

	Blank OD	Concentration of Streblus asper Lour (Bark)					
		1 µg/ml	5 µg/ml	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml
Absorbtion-1	1.222	0.646	0.628	0.563	0.556	0.551	0.399
Absorption-2	1.222	0.646	0.614	0.571	0.568	0.555	0.443
Absorption-3	1.216	0.647	0.615	0.576	0.564	0.560	0.464
Average Absorption	1.22	0.646	0.619	0.568	0.562	0.555	0.435
% Inhibition		47.05	49.26	53.44	53.93	54.51	64.34
log concentration		0	0.69	1	1.69	2	2.69

Chloroform : Methanol : Water = 40:10:1 (Polar solvent)

Figure 1: DPPH sprayed TLC plate for qualitative analysis of antioxidant action of the etahanolic extract S*treblus asper* Lour. leaves and bark

RESULTS

Analgesic activity

The ethanolic extract of bark and leaf of *Streblus asper* Lour. were subjected to acetic acid induced writhing method in mice for preliminary analgesic activity screening showed that the ethanol extract possess analgesic depending upon the nature of their active ingredients in extracts. The ethanol extract of *Streblus asper* leaf & bark, given orally at the dose of 250 and 500 mg/kg, significantly and dose dependently reduced the frequency of acetic acid induced writhing in mice. The crude extract of leaf of 500 mg/kg & 250 mg/kg body weight produced 65.46% & 27.79% inhibition of writhing (Table 1). On the other hand bark of 500mg/kg & 250mg/kg body weight produced 58.70 % & 20.26% inhibition of writhing respectively (Table 2); while the standard drug Diclofenac inhibition was found to 64.94 % at a dose of 25 mg/kg body weight. At the dose of 500 mg/kg, the bark and leaf extract demonstrated statistically significant result ($p<0.01$). The dose of 250 mg/kg of leaf and bark did not show significant result which were ($p<0.01$) and ($p<0.02$) respectively.

Antioxidant activity

In Qualitative analysis yellow color was formed which indicates the plant extracts has the antioxidant property (Figure 1). In Quantitative analysis, average absorption of 1, 5, 10, 50, 100 & 500 concentration of *Streblus asper* Lour. leaf was found 0.257, 0.247, 0.245, 0.194, 0.135, 0.127 respectively and percentage of inhibition found 50, 51.94, 52.33, 62.26, 73.74 & 75.29 respectively (Table 4). In case of 1, 5, 10, 50, 100 and 500 μg/ml concentration of *Streblus asper* Lour. bark the absorption was found 0.646, 0.619, 0.568, 0.562, 0.555 & 0.435 respectively and percentage of inhibition found 47.05, 49.26, 53.44, 53.93, 54.51 & 64.34 respectively (Table 5) whereas in case of 1, 5, 10, 50, 100 & 500 μg/ml concentration of Ascorbic acid the absorption was found 0.246, 0.043, 0.023, 0.017, 0.013 & 0.013 respectively and percentage of inhibition found 53.76, 91.92, 95.68, 96.80, 97.56 & 97.56 respectively (Table 3). From the graph it is found that IC_{50} of the *Streblus asper* Lour. is 1 μg/ml for leaf & 10 μg/ml for bark.

DISCUSSION

Plants are employed as important source of medication in many traditional medications[17, 18]. *Streblus asper* is a rich source of cardiac glycosides. Reichstein and co-workers[19–22] have isolated more than 20 cardiac glycosides from the root bark of *Streblus asper* Lour. and were able to structurally characterize 15 such compounds, mainly as a result of the application of degradative techniques, namely Kamloside, Asperoside, Strebloside, Indroside, Cannodimemoside, Strophalloside, Strophanolloside, 16-O-acetylglucogitomethoside, Glucogitodimethoside, Glucokamloside, Sarmethoside and Glucostrebloside. The other glycosides reported from the roots include b sitosterol-3-O-b-d-arabinofuranosyl- O-a-l-rhamnopyranosyl-O-b-d-glucopyranoside[23], lupanol-3-O-b-d-glucopyranosyl-[1-5]-O-b-d-xylofuranoside[24] and Vijaloside, i.e. periplogenin-3-O-b-d-glucopyranosyl-[1-5]-O-b-d-xylopyranoside[25]. From the stem bark of this plant, a-amyrin acetate, lupeol acetate, b-sitosterol, a-amyrin, lupeol and diol[26], strebloside and mansonin[27] have been isolated. A pregnane glycoside named sioraside[28] has also been isolated. n-Triacontane, tetraiacontan-3-one, b-sitosterol, stigmasterol, betulin and oleanolic acid were identified from the aerial parts[29]. An unidentified cardenolide[30], b-sitosterol, a-amyrin and lupeol were isolated from root bark and leaves[31]. The volatile oil from fresh leaves of S. asper was obtained in 0.005% yield as a brown liquid. The major constituents of the volatile oil were phytol (45.1%), a-farnesene (6.4%), trans-farnesyl acetate (5.8%), caryophyllene (4.9%) and trans-trans-a-farnesene (2.0%). The other constituents were a-copaene, b-elemene, caryophyllene, geranyl acetone, germacrene, d-cadinene, caryophyllene oxide and 8-heptadecene.

Analgesic activity of the ethanol extract of leaf and bark of *Streblus asper* Lour. was tested by acetic acid-induced writhing model in mice. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings. Increased levels of PGE2 and PGF2α in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid. The extract produced significant writhing inhibition comparable to the standard drug Diclofenac sodium. The polar compounds present in the plant extract may be responsible for the obtained analgesic activity. Based on this result it can be concluded that the ethanol extract of both leaf and bark of *Streblus asper* Lour. possess good analgesic activity.

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction.[32] Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases.[33,34]. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals[35]. These components includes, Nutrient-derived antioxidants like ascorbic acid (vitamin C), Tocopherols and Tocotrienols (vitamin E), Carotenoids, and other low molecular weight compounds such as glutathione and Lipoic acid. Antioxidant enzymes, e.g., superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions. Metal binding proteins, such as Ferritin, Lactoferrin, Albumin, and Ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions. Numerous other antioxidant

phytonutrients present in a wide variety of plant foods. The antioxidant property of ethanolic extract of leaf & bark of *Streblus asper* Lour. was assessed by DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity and the result was comparable to the standard ascorbic acid.

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