International Journal of Pharmacy

Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

EVALUATION OF ANTINOCICEPTIVE AND ANTIOXIDANT PROPERTIES OF THE ETHANOLIC EXTRACT OF *SIDA CORDIFOLIA* ROOT FROM BANGLADESH

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ABSTRACT

The crude ethanolic extract of the roots of *Sida cordifolia* Linn. (Family: Malvaceae) was evaluated for its possible antinociceptive and antioxidant properties growing in southeast part of Bangladesh. The ethanolic extract of roots of *Sida cordifolia* exhibited statistically significant ($p>0.001$) writhing inhibition in acetic acid induced writhing model in white albino mice (Swiss-webstar strain). The crude extract produced 25.77% inhibition of writhing at the dose of 250 mg/kg body weight & 44.11 % inhibition of writhing at the dose of 500 mg/kg body weight while the standard drug diclofenac inhibition was found to 51.97 % at a dose of 25 mg/kg body weight. The antioxidant property of ethanolic extract of *Sida cordifolia* was assessed by DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity. In DPPH scavenging assay the IC_{50} value was found to be (65 µg/ml) which was comparable to the standard ascorbic acid (16 µg/ml). Phytochemical nature (group determination of plant constituent) and selected phytochemical analysis of the ethanolic extract of the roots of *Sida cordifolia* indicated the presence of steroid, reducing sugars, tannin & saponin types of compounds. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Keywords: *Sida cordifolia*, antinociceptive, acetic acid induced writhing model, DPPH free-radical scavenging.

INTRODUCTION

Sida cordifolia Linn. (Family: Malvaceae) is popularly known as "Country mallow" and "Bala" found along roadsides throughout the tropical and subtropical plains of Bangladesh, India and Sri Lanka. It's synonym is *S .herbacea, S. althaeitolia, S. rotundifolia*. The leaves of the plant are reported to possess analgesic, anti-inflammatory [1], anticancer [2], diuretic, laxative, hypoglycemic [3] and hepatoprotective [4] activities. Further, studies showed that aqueous fraction of hydroalcoholic extract of leaves induce vasorelaxation [5], hypotension and bradycardia [6]. It is also considered as an excellent anti-oxidant activity [7]. This plant is used in folk medicine for the treatment of stomatitis, blenorrhea, asthmatic bronchitis, and nasal congestion [8]. Phytochemical studies of its roots have shown the presence of ephedrine, vasicinol, vasicinone and N-methyl tryptophan [9]. Because of ephedrine, various ayurvadic preparation of this plant used in asthma, fat lose, increase energy [10].

The plant contains mainly alkaloids, fatty oils, steroids, resin, resin acids, mucin and potassium nitrate. The demulcent and laxative effects are reported with the seeds and it is useful for bowel complaints. The root was found to possess astringent, diuretic and tonic properties. The root is administered in nervous disorders such as hemiplegia, facial paralysis and in urinary disorders [11]. Further, studies showed that aqueous fraction of hydroalcoholic

extract of leaves induce vasorelaxation [12]. Cancer and atherosclerosis, two major causes of death, are salient "free radical" diseases in human. Reactive oxygen species (ROS) have a tendency to donate oxygen to other substances. Many such reactive species are free radicals and have a surplus of one or more free-floating electrons rather than having matched pairs and are, therefore, unstable and highly reactive includes the hydroxyl radical (OH.), the superoxide radical (O.2), the nitric oxide radical (NO.) and the lipid peroxyl radical (LOO.) cause severely deleterious effects on the human body [13].

Enzymatic and non-enzymatic reactions like respiratory chain reaction, the phagocytosis, prostaglandin synthesis, cytochrome P450 system and oxidative phosphorylation (i.e. aerobic respiration) in the mitochondria [14]. ROS are the products of normal cellular metabolism, having both deleterious and beneficial effect in the body [15].

The balance between the production of free radicals and the antioxidant defenses in the body has important health implications. If there are too many free radicals produced and too few antioxidants, a condition of "oxidative stress" develops which may cause chronic damage body [15]. Antioxidants play an excellent role in preventing cell damage. They donate their own electrons to free radicals. Free radical accepts the electron from antioxidant and they do not attack the cell and the chain reaction of oxidation is inhibited [16]. Phenolic compounds, flavonoid and triterpenoids containing foods and beverages with antioxidant activity have been reported [17]. Very recent, health risks and toxicity have been reported using synthetic antioxidants restricted [18]. Some well known natural antioxidants like rosemary and sage are already exploited commercially either as antioxidant additives or as nutritional supplements stipulating the antioxidant potential of plant species [19].

In recent years, the interest in natural antioxidant, especially of plant origin, has greatly increased [20]. Pain is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause [21].

Analgesic activities are commonly exhibited by the non-steroidal anti-inflammatory drugs (NSAIDS). These NSAIDs exert anti-inflammatory effect principally by inhibiting the synthesis of prostaglandin [22]. Since no literature is currently available to substantiate antinociceptive and antioxidant activities from ethanolic extract of *Sida cordifolia* root, therefore the present study is a part of our on-going pharmacological and chemical screening of selected *Sida cordifolia* root and designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antinociceptive and antioxidant activities.

MATERIALS AND METHODS

Collection and identification of plant materials: For this present investigation the *Sida cordifolia* Linn was collected from Khulna region, Bangladesh in November, 2010. The plant was identified by Bangladesh National Herbarium, Mirpur, Dhaka. (Access no is - 31116).

Preparation of ethanolic extract: About 600 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 7 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. The filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract.

Drug: Drug employed in the study were: diclofenac sodium (Opsonin Chemical Industries Ltd, Bangladesh).

Chemicals: 1,1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid were purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA) and other chemicals were of analytical grade.

Preparation of plant extract: The plant material was shade dried with occasional shifting and then powdered with a mechanical grinder, and stored in a tight container. The dried powder (1.5 kg) was refluxed with ethanol for three hours. The total filtrate was concentrated to dryness, in vacuo at 40°C to render the ethanol extract for investigation.

Animal: For the experiment, twenty swiss albino mice of either sex, weighing between 20-25 g, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). Animals were maintained under standard environmental conditions (temperature: (24.0 ± 1.0°C), relative humidity: 55-65% and 12 h light/ dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal

experiment were approved by the institutional animal ethical committee.

Phytochemical screening: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the Dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, and steroids with Libermann-Burchard reagent. Reducing sugars with Benedict's reagent [23,24,25].

Antinociceptive activity: Antinociceptive activity of the crude extract was tested using the model of acetic acid induced writhing in mice [26, 27]. The experimental animals were randomly divided into three 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 was test groups and was treated with the extracts at dose 500 mg/kg of body weight respectively. Each mouse was weighed properly and the dose of the test samples and control materials were adjusted accordingly. Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection in peritoneum. Then after an interval of 10 min, the number of writhes (squirms) was counted for 5 min.

Screening for In-vitro Anti-oxidant Activity

Free radical scavenging activity by DPPH Method: Quantitative assay was performed on the basis of the modified method of Choi et al [28]. Stock solutions (10 mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 mg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated. The experiment was performed in duplicate and average absorption was noted for each concentration. Ascorbic acid was used as positive control

RESULTS

Preliminary phytochemical analysis: Results of different chemical tests on the methanol crude leaves extract of *S. cordifolia* showed the presence of Steroid, Reducing sugars, Tannins and Saponin (Table-1).

Antinoceptive: The results of the test showed that *S. cordifolia* Linn. ethanol extract 500 mg/kg exhibit highly significant (P<.001) inhibition of writhing reflex by 44.30 % & 250 mg/kg showed 25.77% inhibition while the standard drug diclofenac inhibition was found to be 45.22% at a dose of 25 mg/kg body weight. The result is showed in table 2. From the above observation it can be suggested that the ethanolic extract of *S. cordifolia* Linn. is an effective analgesic that supports the claim about the root being used as an analgesic in traditional practice. However further study should be done for its isolated, purified active principles.

Antioxidant: DPPH applied TLC plates ware observed under UV detector both in short (254 nm) and long (360 nm) wavelength. Antioxidant components in the ethanolic extract of *S. cordifolia* were identified. EeOH extract of *S. cordifolia* showed potential antioxidant activity) (Figure-1) where the IC_{50} was 65 ± 0.49 µg mL^{-1} ($P < 0.001$), as compared to that of ascorbic acid (IC_{50} 16 ± 0.21 µg mL^{-1}) ($P < 0.001$) which is a well known antioxidant. The extract caused an increase in DPPH free radical scavenging activity (% inhibition) as increasing dose (Figure-1).

DISCUSSION

Preliminary phytochemical screening showed the presence of Flavonoid, tanin, alkaloid in the plant extract. Multiple biological effects, including antioxidant activity commonly found in plants containing Polyphenolic compounds, like flavonoids, tannins and phenolic acids. [29] Tannic acid present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action. It was shown that the percentage (%) scavenging of DPPH radical was increased significantly with increasing dose, $P< 0.001$. IC_{50} value of the extract was found to be very fairly significant (65 ± 0.49 µg/ml) when compared to the IC_{50} value of the reference compounds ascorbic acid and BHA (16 ± 0.21 µg/ml) respectively. Antinociceptive activity of the ethanol extract of *S. cordifolia* 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 .[30] Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be

responsible for pain sensation caused by intraperitoneal administration of acetic acid . [31] The extract produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 2). The polar compounds present in the plant extract may be responsible for the obtained antinociceptive activity. Based on this result it can be concluded that the ethanol extract of *S. cordifolia* might possess antinociceptive activity). Tannins are important compounds known to be potent cyclooxygenase-1 inhibitors and with anti-phlogistic activity. [32] The plant is also reported to contain saponins. There is growing interest in natural saponins caused as much by the scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of pharmacological activities; for instance, bactericidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anti-cancer and antiallergic. [33]

CONCLUSION

In conclusion it can be revealed that the crude ethanolic extract of *S. cordifolia* root possess significant antinociceptive as well as antioxidant activities. The potential of the extract of *S. cordifolia* as antinociceptive and antioxidant agents may be due to the presence of phytoconstituents like tannins, phenolics etc and might be responsible for its activity and justify its use as a traditional folk remedy. However, extensive researches are necessary to search for active principles responsible for these activities.

Table 1: Results of different group tests of ethanolic extract of *S. cordifolia* roots.

Plant Extract	Alkaloid	Reducing Sugars	Tannins	Gums	Flavonoids	Saponin	Steroid
EE	-	+	+	-	-	+	+

EE: Ethanol extract of S. cordifolia; +: Positive result; - : Negative result

Table 2: Effects of *S. cordifolia* spike extract on writhing effect on acetic acid induced mice.

Treatment	Dose	Mean Writhing± SEM	% writhing	% writhing inhibition
Test group1 Distilled water	------	22.9±0.60*	100	--
Test group 2 Positive control	25mg/kg	11.0±0.22*	48.03%	51.97
Et extract of *S. cordifolia*	250 mg/kg	17.0±0.47*	74.23	25.77
Et extract of *S. cordifolia*	500 mg/kg	12.08±0.60*	55.89	44.11

*Values are expressed as mean±SEM (Standard Error Mean); Et.: Ethanolic; * indicates P < 0.001, one-way ANOVA followed by Dunnet's test as compared to control.*

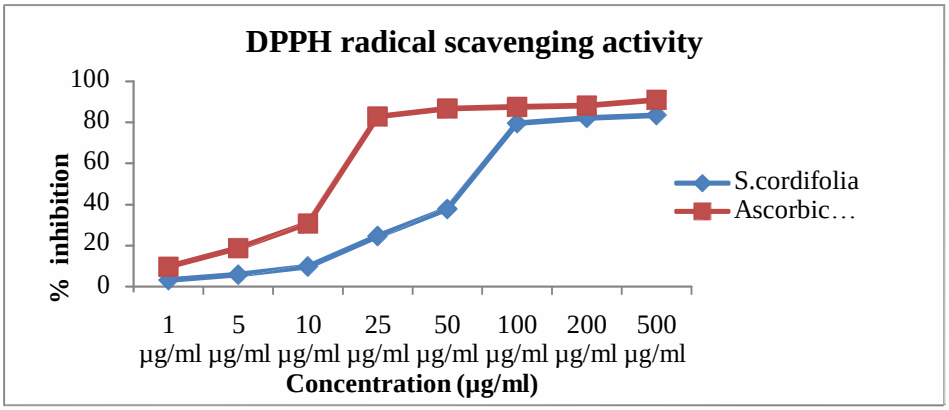

Figure-1: DPPH radical scavenging activity of the ethanolic extract of *Sida cordifolia* and standard.

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