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(Research Article)

Evaluation of Antinociceptive and Antioxidant Potential from the Leaves of *Spilanthes paniculata* Growing in Bangladesh

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ABSTRACT

The crude ethanolic extract of the leaves of Spilanthes paniculata Wall.ex DC (Family: Asteraceae) was evaluated for its possible antinociceptive and antioxidant activities abundantly growing in northeast part of Bangladesh. At the dose 500 mg/kg body weight, the extract showed a significant analgesic activity in acetic acid induced writhing in mice showing 37.61% inhibition (P<0.001) comparable to that produced by Diclofenac Na (45.02%) used as standard drug. Five complementary test systems, namely DPPH free radical scavenging, nitric oxide scavenging, reducing power, Fe^{++} ion chelating ability and total phenolic content were used for determining antioxidant activities of the leaf extract. In DPPH free radical scavenging test, IC_{50} value for ethanolic crude extract was found fairly significant 36.25 µg/ml while compared to the IC_{50} value of the reference standards ascorbic acid and Butylated Hydroxy Anisole (BHA) (3.93 & 7.10 µg/ml) respectively. The ethanol extract showed maximum nitric oxide (NO) scavenging activity of 70.68% at 100 µg/ml, where as ascorbic acid exhibited 83.83% inhibition at the same concentration. The IC_{50} value for nitric oxide scavenging test was also found significant (73.26 µg/ml) while compared to the IC_{50} value of the reference standard ascorbic acid (37.93 µg/ml). The maximum absorbance for reducing power assay was found to 1.531 at 100 µg/ml while compared to 2.811 and 2.031 for standard ascorbic acid and BHA respectively. The IC_{50} value of the extract as percentage of Fe^{++}ion chelating ability was determined as 18.68 µg/ml where that of EDTA showed 8.87 µg/ml. The total phenolic amount was also calculated as quite high in ethanolic crude extract 278.47 mg/g of gallic acid equivalent. Therefore, the obtained results tend to suggest the antinociceptive and antioxidant activities of the crude ethanolic extract of the leaves of Spilanthes paniculata and justify its use in folkloric remedies.

Key Words: *Spilanthes paniculata*, Antinociceptive, DPPH, Free-radical scavenging, Nitric oxide scavenging, Reducing power, Total phenolic

INTRODUCTION

Spilanthes paniculata (*S. paniculata*) is an important medicinal plant with rich source of therapeutic and medicinal constituents. The genus *Spilanthes* (Asteraceae) comprises 30 species and 9 additional intraspecific taxa that are mainly distributed in the tropical and subtropical regions around the world[1]. This species is famous as a folklore remedy for toothache and for throat and gum infections, earning it the English nickname, the "toothache plant". *S. paniculata* all showed larvicidal activity against Anopheles mosquitoes suggesting a possible role for *Spilanthes* in not just the treatment but also prevention of malaria[2]. *Spilanthes* contains a number of biologically active compounds[3], of which the most studied have been the alkylamides[4]. Isolated alkylamides from *Spilanthes* have demonstrated activity against mosquito larvae. Although there are no

published reports of antiplasmodial activity of isolated *Spilanthes* alkylamides, alkylamides from other plants have shown such activity[5]. Roots of *S. paniculata* release more than 90% of N, P and K within 150 day. *S. paniculata* can play a significant role in soil nutrient enrichment in poorly managed shifting cultivation systems[6].

Pain is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause[7]. Excessive generation of reactive oxygen species (ROS) and other radicals can damage proteins, carbohydrates, polyunsaturated fatty acids, and DNA, and may thus lead to oxidative stress and to a variety of degenerative processes and diseases such as aging, immunodeficiencies, neurologic disorders, inflammation, arthritis, ischemia, arteriosclerosis, coronary heart disease, stroke, diabetes mellitus, Parkinson's disease, Alzheimer's disease and certain cancers.[8-14] ROS are continuously produced during normal physiologic events and removed by antioxidant defense mechanisms.[15] Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytoconstituents due to their well known abilities to scavenge free radicals(i.e. antioxidant power).[15]

Since no literature is currently available to substantiate antinociceptive and antioxidant activities from ethanolic extract of *S. paniculata* leaves growing in Bangladesh, therefore the present study was designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antinociceptive and antioxidant activities that also confirm its use as pain killer and other pathological conditions where free radicals are implicated.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The plant (leaves) *S. paniculata* was collected at December, 2010 from Noakhali, northeast distrct of Bangladesh and was identified by Bangladesh National Herbarium, Mirpur, Dhaka. (Accession number-DACB-39538).

Preparation of Ethanolic Extract

The leaves of *S. paniculata* were freed from any of the foreign materials. Then the plant materials were chopped and air-dried under shed temperature followed by drying in an electric oven at 40º C. The dried plant materials were then ground into powder. About 600g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1.5 liters of 80% ethanol. The container with its contents was sealed and kept for a period of 4 days accompanying occasional shaking and stirring. The ethanolic extract was filtered by Buchner funnel and the filtrate was concentrated with rotary evaporator at bath temperature not exceeding 40˚ to have gummy concentrate of greenish black extract (Yield approx. 16.55%).

Test for Different Chemical Groups

The crude ethanolic extract was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins.[16] In each test 10% (w/v) solution of the extract in ethanol was taken.

Test Animals and Drug

Young Swiss-albino mice either sex, 3-4 weeks of age, weighing 20-25g, were used for in vivo pharmacological screening. Mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were housed in standard environmental conditions at animal house of Chittagong Laboratories, BCSIR, Chittagong and fed with rodent diet and water ad libitum. The ethics for use of experimental animals were followed carefully.

The standard drug Diclofenac sodium was used for this study and purchased from Square Pharmaceuticals Ltd, Bangladesh.

Chemicals

1,1-Diphenyl-2-picryl hydrazyl (DPPH), L-ascorbic acid, BHA (Butylated Hydroxy Anisole), Gallic acid, Folin-ciocalteu phenol reagent, Ferrozine and Griess reagent were obtained from Sigma Chemical Co.[(St. Louis, MO, USA)]. Trichloroacetic acid (TCA), Phosphate buffer (pH 6.6), Potassium ferricyanide [$K_3Fe(CN)_6$], $FeCl_2$, $FeCl_3$, Sodium nitroprusside, Ethanol, Sodium phosphate, EDTA, Tween 80, Ammonium molybdate and Sodium carbonate were of analytical grade and purchased from Merck (Darmstat, Germany).

Antinociceptive Activity

The antinociceptive activity of the crude ethanolic extract of *S. paniculata* was studied using acetic acid induced writhing model in mice[17,18]. The animals were divided into control, positive control and test groups with five mice in each group. The animals of test groups received test substance at the dose of 250 and 500 mg/kg body weight. Positive control group was administered with Diclofenac sodium (standard drug) at the dose of 25 mg/kg body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10ml/kg body weight.Test samples, standard drug and control vehicle were administered orally 30min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed writhing (constriction of abdomen, turning of trunk and extension of hind legs) for 5 min.

Antioxidant Activities

DPPH free radical scavenging activity

The method of Chang *et al*.[19] was used for performing the DPPH radical scavenging activity. A stock solution (5mg/ml) of ethanolic extract of *S. paniculata* (5 mg/ml) was prepared in respective solvent systems. A serial dilutions were the carried out to obtain concentrations of 5, 10, 20, 40, 60, 80, 100 µg/ml. An equal amount of sample solution was mixed with an equal amount of 0.1 mM methanolic solution of DPPH, The mixture was vortex and allowed to stand at the dark at 25 °C for 30 min. After 30 min incubation, the absorbance of the mixture was read against a blank at 517 nm using a double beam Analykjena UV/Visible spectrophotometer (Model 205, Jena, Germany). The radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

$$I\ (\%) = (Ablank - Asample / Ablank) \times 100$$

Where Ablank is the absorbance of the control (containing all reagents except the test compound), and Asample is the absorbance of the experimental sample with all reagents. IC_{50} value (the concentration of sample required to scavenge 50% DPPH free radical) was calculated from the plot of inhibition (%) against the concentration of the extract. All determination was carried out in triplicate and average of the results was noted. Ascorbic acid and BHA was used as standard for this study.

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically[20]. Sodium nitroprusside (5 mmol) in phosphate buffered saline was mixed with different concentrations of the extract (5–100 µg/ml) dissolved in methanol and incubated at 25 °C for 30 min. In control there was not test sample but an equivalent amount of methanol was used. After 30 min, 1.5 ml of incubation the solution was taken and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm with a double beam Analykjena UV/Visible spectrophotometer (Model 205, Jena, Germany). The nitric oxide (NO) radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

$$I\ (\%) = (Ablank - Asample / Ablank) \times 100$$

Where Ablank is the absorbance of the control reaction (containing all reagents except the test compound), and Asample is the absorbance of the experimental sample with all reagents. IC_{50} value is the concentration of sample required to scavenge 50% nitric oxide free radical and was calculated from the plot of inhibition (%) against the extract concentration. All the determinations were carried out in triplicate and average of the absorptions was noted. Ascorbic acid was used as positive control standard for this study.

Reducing power assay

The method of Dehpour *et al*.[21] was followed to determine the reducing power of *S. paniculata* leaves of ethanolic extract. Different concentrations of the extract (5-100 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was then incubated at 50°C for 20 min and a 10% solution of trichloroacetic acid (2.5 ml) was added to it. It was then centrifuged at 3000 rpm for 10 min. The upper layer of the mixture (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml, 0.1% $FeCl_3$ and the absorbance of the mixture was measured at 700 nm with the same spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power.

All the determinations were carried out thrice and average of the results was taken. Ascorbic acid and BHA were used as the standard reference compounds in this study.

Ferrous ion chelating ability

The ferrous ions chelating activity of ethanol extract and standards were investigated according to the method of Dinis *et al*.[22]. Briefly, different concentrations of the extract (5-100 µg/ml) were added to 0.1ml solution of 2 mM ferrous chloride ($FeCl_2$). Then, the reaction was initiated by the addition of 0.2ml of 5 mM Ferrozine and mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm in spectrophotometer, wherein the Fe^{+2} chelating ability of extracts was monitored by measuring the ferrous ion-Ferrozine complex. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given in the below formula:

$$\text{Ferrous ions chelating ability (\%)} = [(A_0 - A) / A_0] \times 100$$

Where A_0 is the absorbance of the control solution (containing all reagents except extract); A is the absorbance in the presence of the sample of plant extracts. All the tests were carried out in triplicate and EDTA was used as standard.

Total phenolic content determination

The modified Folin-Ciocaltu method[23] followed to determine the total phenolic content of the extract. A 0.5 ml of each extract (1 mg/ml) was mixed with 5 ml Folin-Ciocaltu reagent (1:10 v/v distilled water) and 4 ml (75g/l) of Sodium carbonate and the mixture was then vortexed for 15 second for the development of color the mixture was allowed to stand for 30 min at 40°C.Then the absorbance was read at 765 nm with the same spectrophotometer. Total phenolic content was calculated as mg of Gallic acid equivalent per gram using the equation obtained from a standard Gallic acid calibration curve y = 6.2548x -0.0925, R^2=0.9962.

Statistical Analysis

For antioxidant determination, data were presented as mean ± Standard deviation (S.D). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the control group. *p* values < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Chemical Group Test

Results of different chemical tests on the ethanolic extract of *S. paniculata* leaves showed the presence of saponins, gums, tannins and significantly presence of flavonoid (Table-1).

Analgesic Activity

Table-2 showed the effect of the ethanolic extract of *S. paniculata* on acetic acid induced writhing in mice. At the dose of 500 mg/kg of body weight, the extract produced 37.61% writhing inhibition in test animals. The results were statistically significant (P <0.001) and was comparable to the standard drug Diclofenac Na, which showed 45.02% at a dose of 25 mg/kg weight. Antinociceptive activity of the ethanolic extract of ethanolic extract of *S. paniculata* leaves was tested by acetic acid induced writhing model in mice. The peripheral analgesic effect of the plant's extract may be mediated via inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators), while the central analgesic action of the extract may be mediated through inhibition of central pain receptors. This hypothesis is in consonance with those of Koster *et al*.[24] and Williamson *et al.*[25] who postulated that acetic acid-induced writhing and hot-plate test methods are useful techniques for the evaluation of peripherally- and centrally-acting analgesic drugs, respectively. With respect to the writhing test, the research group of Deraedt *et al.* described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid.[26] These authors found high levels of prostaglandins PGE_2 and $PGF_{2\alpha}$ during the first 30 min after acetic acid injection. On the basis of the result of acetic acid induced writhing test, it can be concluded that the ethanolic extract of *S. paniculata* might possess an antinociceptive activity.

Antioxidant Activities

Ethanolic extract of *S. paniculata* was screened for evaluation of its possible antioxidant activities. Five complementary test systems, namely DPPH free radical scavenging, nitric oxide scavenging activity, reducing power, ferrous ion chelating ability and total phenolic contents determination were followed for this analysis.

DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity of the *S. paniculata* was found to be increased with the increase of concentration of the extract. The extract exhibited 88.52±0.087% radical inhibitions at 100 µg/ml whereas at the same concentration the standards ascorbic acid and BHA exhibited 95.86 ±0.031 and 93.09 ±0.019% inhibitions respectively. IC_{50} value of the extract was found to be very fairly significant (36.25±0.091 µg/ml). When compared to the IC_{50} value of the reference compounds ascorbic acid and BHA (3.93 ± 0.027 and 7.10 ± 0.035 µg/ml) respectively. A method based on the scavenging of the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has been used extensively to predict the antioxidant activities of extracts of plants[27,28]. The high inhibition value of *S. paniculata* ethanol extract may due to the presence of tannins and significant amount of flavonoids in the extract as phytochemicals. Tannins and Flavonoids, commonly found in plants have been reported to have significant antioxidant activity[29].

Nitric oxide (NO) Scavenging Assay

The scavenging of NO by the ethanol extract of *S. paniculata* was increased in dose dependent manner. Table-4 illustrates a significant decrease in the NO radical due to the scavenging ability of the extract and ascorbic acid. The ethanol extract showed maximum scavenging activity of 70.68±0.58% at 100 µg /ml, where as ascorbic acid at the same concentration exhibited 83.83±0.91% inhibition. The IC_{50} value for ethanolic extract was found fairly significant (73.26±0.96 µg/ml) while compared to the IC_{50} value of the reference standard ascorbic acid (37.93±0.83 µg/ml). NO scavenging capacity of the extract may help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric oxide is also implicated for inflammation, cancer and other pathological conditions[30]. NO works as a atypical neural modulator that is involved in neurotransmitter release, neuronal excitability and learning and memory. Besides its role in physiologic processes, it also participates in pathogenic pathways underlying a large group of disorders including muscle diseases, inflammatory bowel disease, sepsis and septic shock, primary headaches and stroke. Additionally, increasing evidence shows that NO modulates neurotoxin induced cell damage and is involved in neuronal cell death in Parkinson's disease (PD) and other neurodegenerative disorders such as Alzheimer disease[31]. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals[32]. Preliminary phytochemical studies of the ethanol extract of *S. paniculata* showed the presence of tannins and significant amount of flavonoids therefore suppression of released NO may be attributed to direct NO scavenging.

Reducing Power Assay

In determination of reducing power of ethanolic crude extract of *S. paniculata* ascorbic acid and BHA was used as positive control (Table 5). The maximum absorbance for ethanolic extract was found to be (1.531 ± 0.021) at 100 µg/ml concentration while compared to standard ascorbic acid (2.8111 ± 0.013) and BHA (2.031 ±0.019) respectively, at the same concentration. With the increase of concentration, the absorbance of the extract was found to be increased and those for the standards were also increased with increasing concentration. A direct correlation between antioxidant capacity and reducing power of certain plant extracts has been reported[33]. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[34]. The maximum absorbance for the ethanolic extract of *S. paniculata* was found to be 1.531 at 100 µg/ml while that of standard ascorbic acid and BHA was found as 2.8111 and 2.031, respectively (Table 5).

Fe^{++} Ion Chelating Ability

Fe^{++} ion chelating ability of ethanol extract is shown in table 6. The extract showed 87.04±0.016% Fe^{++} ion chelating ability at 100 µg/ml where as the standard EDTA showed 99.75±0.011% at the same concentration. The IC_{50} value of the extract was also found significant (18.68±0.019 µg/ml) while compared to the IC_{50} value of the reference standard EDTA (8.87 ± 0.035 µg/ml). Bivalent transition metal ions (e.g. Fe^{++}) play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry[35]. These processes can be delayed by iron chelation. Iron can generate free radicals from peroxides and may be implicated in human cardiovascular disease[36]. Therefore, minimizing its concentration affords protection against oxidative damage. Ferrozine can quantitatively form complexes with Fe^{2+}. The absorbance of Fe^{2+}-ferrozine complex was decreased dose-

dependently, that is, the activity was increased on increasing concentration from 5 to 100 µg/ml. Table 5 is exhibiting the comparative percentage Fe^{++} ion chelating ability of ethanol extract and standard compound (Na_2EDTA). The IC_{50} value of extract as percentage (%) Fe^{++}ion chelating ability was determined as 18.68±0.019 µg/ml where Na_2EDTA showed 8.87±0.035 µg/ml.

Total Phenolic Content

The amount of total phenolic content was calculated as quite high in the ethanolic crude extract of *S. paniculata* (278.47±0.61 mg/g of gallic acid equivalent) (Table 7). Phytochemical components, especially phenolic compounds (such as flavonoids, phyenyl propanoids, phenolic acids, tannins etc.) are very important components for the free radical scavenging and antioxidant activities of plants. Polyphenols are generally of the chemical patterns; phenolic groups react as hydrogen donors and neutralize the free radicals [28,33]. In the present study the total amount of phenolic compounds was calculated as quite high in the ethanol extract of *S. paniculata* leaves. The result of present study revealed that the presence of high concentration of phenolic components in the extract might cause the high inhibition value of the extract. Phenols are important components of plants. It is reported that the hydroxyl group of the phenolic compounds to eliminate radicals and they contribute directly to antioxidant effect of the system [34].

CONCLUSION

In conclusion it can be revealed that the crude ethanolic extract of *S. paniculata* leaves possess significant antinociceptive as well as antioxidant activities. The potential of the extract of *S. paniculata* as antinociceptive and antioxidant agents may be due to the presence of phytoconstituents like tannins, flavonoids, phenolics etc and might be responsible for its activity and justify its use as a traditional folk remedy for toothache. 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. paniculata* leaves

Phytoconstituents	Ethanol extract of *S. paniculata*
Alkaloid	-
Reducing sugars	-
Tannins	+
Gums	+
Flavonoids	++
Saponin	+
Steroid	-

+: Positive result; - : Negative result; ++: significantly positive

Table 2: Effects of the ethanolic extract of *S. paniculata* on acetic acid induced writhing of mice (n=5)

Group	*Treatment and Dose*	*Number of writhes (% Writhing)*	% Writhing Inhibition
Control	1% tween 80 solution 10 ml/kg, p.o.	12.95± 0.68 (100)	---
Positive Control	Diclofenac Na 25 mg/kg, p.o.	7.12± 0.57 * (54.98)	45.02
Test Group- 1	Et. Extract of *S. paniculata* 250 mg/kg, p.o.	10.19± 0.93 * (78.68)	21.31
Test group- 2	Et. Extract of *S. paniculata* 500 mg/kg, p.o.	8.08± 0.58 ** (62.39)	37.61

*Values are expressed as mean±SEM (Standard Error Mean); Et.: Ethanolic; * indicates P < 0.01; ** indicates P < 0.001, one-way ANOVA followed by Dunnet's test as compared to control; n = Number of mice; p.o.: per oral.*

Table 3: DPPH radical scavenging activity of the ethanolic extract of *S. paniculata* leaves and standards

Concentration (µg/ml)	% Inhibition of ethanol extract and Standards at different concentration		
	Ethanol Extract of *S. paniculata* leaves	Ascorbic acid (standard)	Butylated Hydroxyanisole (BHA) (standard)
5	28.37±0.091	69.44 ±0.021	53.88 ±0.028
10	36.66±0.088	79.29 ±0.291	77.23 ±0.011
20	46.67±0.041	83.96 ±0.011	90.18 ±0.018
40	55.18±0.090	91.98 ±0.019	91.11 ±0.009
60	70.21±0.052	95.28 ±0.033	92.03 ±0.013
80	81.17±0.070	95.58 ±0.017	92.31 ±0.021
100	88.52±0.087	95.86 ±0.031	93.09 ±0.019
IC_{50} (µg/ml)	36.25±0.091	3.93±0.027	7.10±0.035

The values are expressed as mean ± standard deviation (n=3).

Table 4: Nitric oxide radical scavenging activity of the ethanolic extract of *S. paniculata* leaves and standard

Concentration (µg/ml)	% NO inhibition of the extract and standard at different concentration	
	Ethanol Extract of *S. paniculata*	Ascorbic acid (standard)
5	10.15 ± 0.96	26.58 ± 0.53
10	23.62 ± 0.42	37.25 ± 0.85
20	31.83 ± 0.97	46.78 ± 0.46
40	39.16 ± 0.64	56.49 ± 0.78
60	48.62 ± 0.86	67.27 ± 0.71
80	57.16 ± 0.67	70.28 ± 0.65
100	70.68 ± 0.58	83.83 ± 0.91
IC_{50} (µg/ml)	73.26 ± 0.96	37.93 ± 0.83

The values are expressed as mean ± standard deviation (n=3).

Table 5: Reducing power assay of the ethanolic extract of *S. paniculata* leaves and standards

Concentration (µg/ml)	Average absorbance at 700nm of extract and Standards at different concentration		
	Ethanol Extract of *S. paniculata* leaves	Ascorbic acid (standard)	Butylated Hydroxyanisole (BHA) (standard)
5	0.135 ±0.010	0.370 ±0.013	0.435 ±0.011
10	0.564 ±0.011	0.820 ±0.017	0.776 ±0.013
20	0.759 ±0.021	1.447 ±0.011	1.598 ±0.012
40	1.053 ±0.013	1.929 ±0.014	1.749 ±0.017
60	1.165 ±0.014	2.624 ±0.015	1.842 ±0.013
80	1.436 ±0.012	2.772 ±0.012	1.976 ±0.015
100	1.531 ±0.021	2.811 ±0.013	2.031 ±0.019

The values are expressed as mean ± standard deviation (n=3).

Table 6: Fe^{2+} ion chelating ability of ethanol extract of *S. paniculata* leaves and EDTA (Standard)

Concentration (µg/ml)	% Chelating Ability of different solvent extract and Standard	
	Ethanol extract of *S. paniculata* leaves	Na_2EDTA (Standard)
5	14.38±0.018	36.97±0.032
10	34.32±0.015	57.71±0.027
20	53.69±0.019	81.69±0.037
40	70.81±0.015	91.35±0.019
60	76.45±0.018	99.19±0.020
80	83.99±0.014	99.30±0.021
100	87.04±0.016	99.75±0.011
IC_{50} (µg/ml)	18.68±0.019	8.87±0.035

The values are expressed as mean ± standard deviation (n=3).

Table 7: Total phenolic content of the ethanol extract of *S. paniculata* leaves

Extract	Avg. absorbance at 765 nm	Total phenolic content
		mg of Gallic acid equivalent (GAE) per g of dry extract
Ethanol extract of *S. paniculata* leaves	1.38±0.078	278.47±0.61

The values are expressed as mean ± standard deviation (n=3).

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