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Short Communication

EVALUATION OF ANTIOXIDANT POTENTIAL OF *SPILANTHES PANICULATA*

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ABSTRACT

Objective: The present study was conducted to evaluate the antioxidant potential of *Spilanthes paniculata*.

Methods: The antioxidant activity of three different extracts (ethanol, methanol and chloroform extracts) of the whole plant was evaluated by determining total antioxidant capacity using phospho molybdenum method and total flavonoid content by Aluminum chloride colorimetric method.

Results: Preliminary results from phytochemical screening showed that ethanol, methanol and chloroform extracts of *S. paniculata* contain flavonoids along with other phyto chemicals in moderate to high amounts. The extracts were then evaluated for total antioxidant capacity using ascorbic acid as the standard. The total antioxidant capacity of ethanol, methanol and chloroform extracts was found to be 153.54±1.343, 157.97±6.713 and 200.38±4.028 mg/g of plant extract which were expressed as Ascorbic Acid Equivalents (AAE). Also total flavonoid content was determined and was expressed as quercetin equivalents (QE). The content of flavonoid compounds in ethanol, methanol and chloroform extract was estimated to be 101.58±55.919, 121±74.318 and 51.07±19.121 mg/g dry weight of extract.

Conclusion: Based on these findings, it can be concluded that the significant antioxidant potential of the extracts might be attributed to moderate to high amount of flavonoids present in the extract.

Keywords: *Spilanthes paniculata,* Phytochemical screening, Flavonoid, Antioxidant.

Plants of the genus *Spilanthes*, mainly distributed in the tropical and subtropical regions around the world, have traditionally been used for the treatment of a number of disease conditions such as malaria [1, 2]. *S. paniculata* (Family-Asteraceae) is an important medicinal plant with the rich source of therapeutic constituents. *Spilanthes* contains a number of biologically active compounds [3], of which the most studied have been the alkyl amides, which *S. paniculata* possesses in abundance [4]. Antioxidants which scavenge active oxygen species (free radicals) are found in a variety of food stuff and are commonly referred to as scavengers. Activated oxygen is thought to be a major factor in aging, hardening of an arteries, diabetes, cancer and tissue injury of skin [5]. Plants are rich source of antioxidants and considerable amount of data has been generated on antioxidant properties of food plants around the globe [6, 7]. However, traditionally used medicinal plants still await such screening [8]. Therefore, as a part of our continuing interest in searching biological activities of tropical plants, this study was aimed to evaluate the antioxidant potential of *S. paniculata* extracts using total antioxidant capacity and total flavonoid content determination assays. To the best of our knowledge, this is the first report of antioxidant activity studied with different extracts of *S. paniculata.*

Our study was initiated with the collection of the plant. The whole plant was collected from the Curzon Hall Premise of Dhaka University Campus, Dhaka, Bangladesh during April, 2010 and was identified by the experts of Bangladesh National Herbarium, Dhaka with the accession no DACB-35032. The whole plant extracts were prepared as described previously with minor modification [9].

For extraction and other purposes, HPLC grade solvents were used. L-ascorbic acid, quercetin and other chemicals were purchased from Hi-Media Lab Ltd, Mumbai, India. The dried powders were extracted by cold extraction with three solvents-chloroform, ethanol and methanol (200 g powder in two l solvent) and kept for a period of three days accompanying occasional shaking and stirring. The mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then these were filtered through Whatman filter paper (Whatman Ltd., England). The filtrate (ethanol, methanol and chloroform extract) obtained was evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 68ºC temperature. It rendered a gummy concentrate of chocolate black color. The gummy concentrate was designated as crude extract which was dried in the freeze drier and preserved at 4 °C. The extracts obtained were then undergone qualitative phytochemical tests for the identification of chemical constituents, such as alkaloids, flavonoids, steroids, cardiac glycosides, saponins, tannins and terpenoids, which were carried out by the methods described by Harborne and Sazada *et. al.* [10, 11].

Phyto chemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Wagner reagent, flavonoids with the use of conc HCl, tannins with 5%, and saponins with ability to produce suds. Gum was tested using Molish reagents and concentrated sulfuric acid, steroids with sulfuric acid, reducing sugar with the use of α-napthol and sulfuric acid and terpenoids with chloroform and concentrated HCl. The results of phytochemical screening are mentioned in table 1.

Table 1: It shows the results of qualitative phytochemical screening of the extracts

Chemical constituents	Ethanol extract	Methanol extract	Chloroform extract
Alkaloids	++	+	+++
Cardiac Glycosides	+++	+++	+++
Flavonoids	+++	++	++
Saponins	+++	++	-
Tannins	+	+++	-
Terpenoids	+++	+++	+++

Symbol (+++), (++) and (+) indicates presence of phytochemicals in higher, moderate and lower amounts respectively; (-) indicates absence of phytochemicals.

The antioxidant activity of the extracts was tested using two methods: total antioxidant capacity and amount of flavonoids. The antioxidant activity of the extracts of *S. paniculata* was evaluated by the phospho molybdenum method according to the procedure of Jamuna *et al.* [12]. Briefly 0.3 ml (the concentration of the solution is 5 µg/ml) of extract was mixed with 3 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 °C for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer (UV-1650PC, Shimadzu, Japan) against blank. A solution containing 3 ml reagent solution and an appropriate volume (0.3 ml) of the same solvent in place of extract was used as the blank. The results are presented as the average and standard deviation of two experiments. The data were analyzed by using SPSS (SPSS Inc, CA, USA). Total antioxidant capacity of the three extracts of *S. paniculata* was calculated using the standard curve of Ascorbic acid prepared at a concentration range of 0 to 200 µg/ml (y = 0.0138x+0.1276; R^2= 0.9881). And was expressed as No. of equivalents of Ascorbic acid per gram of plant extract using the formula: A = c x v/m, where

A = Total antioxidant capacity, mg/g of plant extract in AAE

c = Concentration of ascorbic acid established from the calibration curve, mg/ml

v= volume of extract in ml

m = weight of ethanol/methanol/chloroform extract of *S. paniculata* in g.

Among the three extracts, chloroform extract was found to possess the highest antioxidant capacity (table 2).

Table 2: It shows the results of total antioxidant capacity of the three extracts

Extract	Total antioxidant capacity (mg/g plant extract) in AAE±SD
Ethanol	153.54±1.343*
Methanol	157.97±6.713*
Chloroform	200.38±4.028 *

Each value is represented as mean±SD (n = 3, *p<0.05 is considered statistically significant), AAE = Ascorbic Acid Equivalents, SD = Standard Deviation

The total flavonoid contents of the extracts were determined according to the colorimetric method [13]. Briefly, each plant extract (0.1 g) was dissolved in 1 ml de ionized water. This solution (0.1 ml) was mixed with 0.2 ml of 10% $AlCl_3.6H_2O$ and 0.2 ml of 1M potassium acetate (CH_3COOK). The mixture was kept at room temperature for 30 min and the absorbance of the reaction mixture was measured at 765 nm. Quercetin was chosen as a standard. Using the standard curve (0–100 µg/ml, y = 0.0098x-0.0366, R^2 = 0.9723), the levels of total flavonoid contents in sample extracts were determined in triplicate. The total flavonoids content is calculated in quercetin equivalents (QE) using the formula: A = (c x v)/m, where

A = total content of flavonoid compounds, mg/g of plant extract in QE

c = concentration of quercetin established from the calibration curve, mg/ml

v = volume of extract in ml

m = weight of chloroform/methanol/ethanol extract of S. paniculata in g.

Flavonoid compounds are commonly found in both edible and inedible plants and plant parts. They have been reported to have multiple biological effects, including antioxidant activity. The content of flavonoid compounds (mg/100g DW) in ethanol, methanol and chloroform extract to be 101.58±55.919, 121±74.318 and 51.07±19.121 Quercetin Equivalents (QE)/g dry weight of extract, Among the three extracts ethanol extract was found to have the highest antioxidant capacity (table 3).

Table 3: It shows the results of total flavonoid content of the three extracts.

Extract	Total flavonoid content (mg/g plant extract) in QE±SD
Ethanol	51.07±19.121*
Methanol	121.73±74.318*
Chloroform	101.58±55.919*

Each value is represented as mean±SD (n = 3, *p<0.05 is considered statistically significant); QE = Quercetin Equivalents, SD = Standard Deviation

Table 4: It shows the mean absorbance values of ascorbic acid and quercetin at different concentrations

Concentration of ascorbic acid (µg/ml)	Absorbance values at 695 nm	Concentration of Quercetin (µg/ml)	Absorbance values at 765 nm
50	1.004	12.5	0.116
100	1.527	25	0.196
150	2.120	50	0.344
200	2.897	100	0.995

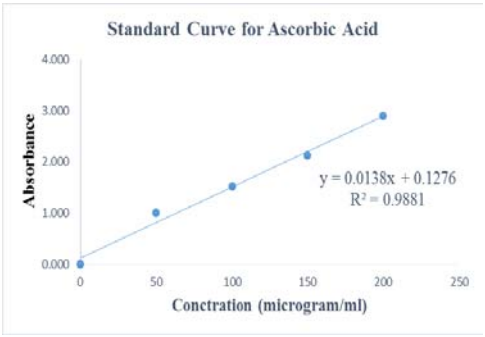

Fig. 1: **It shows ascorbic acid standard curve**

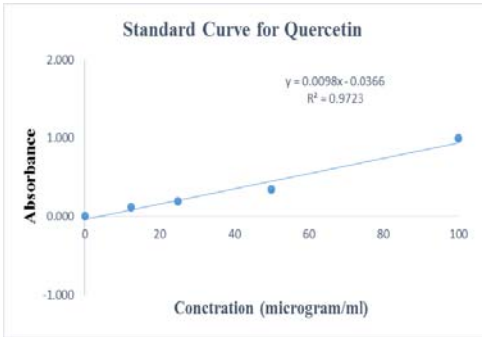

Fig. 2: It shows quercetin standard curve

Many synthetic antioxidant components have shown toxic and/or mutagenic effects. There is a widespread agreement that the synthetic antioxidants such as butyl hydroxy anisole (BHA) and butyl hydroxy toluene (BHT) need to be replaced because of their potential health risks and toxicity [14]. Therefore the attention now has been shifted towards naturally occurring antioxidants.

The present study was conducted to investigate the antioxidant potential of *S. paniculata.* Preliminary phytochemical screening of the different extracts of the plant revealed the presence of various bioactive components of which alkaloid, cardiac glycosides, terpenoid and tannins were the most prominent. Phenolic natural products such as flavonoids are of particular interest because of their antioxidant activity through scavenging oxygen radicals and inhibiting peroxidation. Antioxidants that scavenge free radicals play an important role in cardiovascular disease, aging, cancer, and inflammatory disorders. In addition, these naturally occurring antioxidants can be formulated to give nutraceuticals, which can help to prevent oxidative damage of cells in the body [15]. Extraction is critical to the recovery of antioxidant phytochemicals. The extraction yield depends on solvent, time and temperature of extraction as well as the chemical nature of the sample [16]. Under the same time and temperature conditions, the solvent used and the chemical property of the sample is the two most important factors. We found that among different extracts ethanol extracts contained the highest amount of flavonoid compounds followed by methanol extract and the chloroform extract. The antioxidant activity of plant extracts varies with assay methods. Therefore, a single assay may be inadequate [17]. For this reason, we cross checked antioxidant activities of various extracts of *S. paniculata* with two antioxidant activity assays based on different mechanisms namely total antioxidant activity using ascorbic acid as standard and total flavonoid content using quercetin as standard. Although both methods showed that the extracts possessed significant antioxidant activity, the extent of activity varied among the extracts. Using ascorbic acid as standard, chloroform fraction showed the highest activity while using quercetin as standard, methanol extract possessed the most significant activity. This discrepancy is difficult to explain at this stage. The different extracts might have different compounds other than the flavonoids which either have intrinsic antioxidant activity or has interfered with the assay methods. Recently Saumya *et al.*, interestingly, have shown that *Panax ginseng* extract, even though having comparatively less amount of flavonoid and phenolic contents than leaf extract of *Lagerstroemia speciosa*, could show potential antioxidant and free radical scavenging activity [18]. It can be concluded the plant *S. paniculata* possessed significant antioxidant activity which is evident from the results of our study. However, further studies are required to elucidate the chemical structure of compounds responsible for bioactivity which are on progress.

CONFLICT OF INTERESTS

Declared None

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