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IN VITRO ANTIOXIDANT, TOTAL PHENOLIC CONTENT AND BRINE SHRIMP LETHALITY STUDIES OF *SYNEDRELLA NODIFLORA*

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ABSTRACT

The antioxidant activity of methanol extract of whole plant of *Synedrella nodiflora* as well as its petroleum ether, carbon tetrachloride, dichloromethane and aqueous soluble partitionates were evaluated by DPPH of (1, 1-diphenyl-2-picrylhydrazyl) and phosphomolybdenum total antioxidant assay and compared with standard antioxidants butylated hydroxytoluene (BHT) and ascorbic acid (ASA). The total phenolic content was also determined and expressed in gallic acid equivalent (mg of GAE/g of sample). A great variance was observed for polyphenol content as well as antioxidant activity (1.574-9.4136 mg GAE/g and DPPH IC_{50} 10.52-31.25 µg/ml) depending on the nature of solvent used to fractionate the crude extract. The result demonstrated that dichloromethane soluble fraction revealed the highest amount of phenolic compounds (9.4136 mg GAE/g) and also had significant free radical scavenging activity (IC_{50} 10.52 µg/ml). A positive correlation was observed between total phenolic content and total antioxidant activity of *S. nodiflora* having correlation coefficient (R^2) of 0.9270. The general toxicity of the extractive was studied by brine shrimp lethality bioassay and from the results (LC_{50} 0.023-0.122 µg/ml), it can be well predicted that the crude extract and the partitionate fractions contain cytotoxic principles and have considerable toxic potencies which supported the insecticidal uses of plant by the indigenous people.

INTRODUCTION: *Synedrella nodiflora* (L) is a flowering herb which belongs to the family Asteraceae. *S. nodiflora* grows well in different environments and mainly found in Bangladesh, India, Japan, Span, China and England. The leaf juice is used in the treatment of itch, eczema, scabies and any type of skin disorders [1], while the whole plant is diuretic and laxative [2]. The anti-inflammatory [3], insecticidal [4], analgesic [5] and anti-feedant [6] activities of the plant have also been reported.

In the present study, the organic soluble materials of methanolic extract of whole plants of *S. nodiflora* were subjected to assays for antioxidant activity in terms of total phenolics content and free radical scavenging activity, and preliminary toxicity studies for the first time.

Attempt has been taken to establish a correlation between the total antioxidant activity and total phenolics content in the extractives.

MATERIALS AND METHODS:

Collection and extraction of plant material: Whole plants of *S. nodiflora* were collected in mid 2009 from the Chittagong University campus. The collected plant materials were chopped, dried and powdered and 900 gm was extracted with 2 .0 litre of methanol at room temperature for 7 days. The extracts was filtered through Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature and pressure. A portion of the concentrated methanol extract (10.0 gm) was partitioned by modified Kupchan method[7] using pet-ether, carbon tetrachloride and dichloromethane and subsequent evaporation of solvents yielded pet-ether (PESN, 3.0 gm), carbon tetrachloride (CTCSN, 3.5 gm), dichloromethane (DCMSN, 1.5 gm) and aqueous (AQSN, 1.0 gm) soluble materials. The residues were then stored in a refrigerator for further investigation.

Phosphomolybdenum Antioxidant Assay: The total antioxidant activity of the extract was evaluated by the phosphomolybdenum assay method [8] which is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acedic condition. A 0.3 ml extract (2 mg/ml) was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and the reaction mixture was incubated at 95^{o}C for 90 min. Then the absorbance of the solution was measured at 695 nm using a UV-Visible spectrophotometer against blank after cooling to room temperature. The antioxidant activity was expressed as the number of milligram equivalents of ascorbic acid (mg of ascorbic acid/100g of plant extract).

Free Radical Scavenging Activity: The free radical scavenging activity (antioxidant capacity) of the plant extractives on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method established by Brand-Williams *et al*., (1995). Here, 2.0 ml of a methanol solution of the sample (extractive/standard) at different concentration (500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min of reaction at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by a UV spectrophotometer. Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$(I\%) = (1 - A_{sample}/A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material), and A_{sample} is the absorbance of the test sample. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted with inhibition percentage against extractive/standard concentration.

Total Phenolics Analysis: The total phenolic content of the extractives was determined with the Folin-Ciocalteau reagent using the method developed by Harbertson and Spayd [9]. To 0.50 ml of each sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteau reagent in water and 2 ml of Na_2CO_3 (7.5%, w/v) were added and incubated at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm using a SPECTRAmax-PLUS384 UV–Vis spectro-photometer. The phenolic content was expressed as milligram of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract.

Brine Shrimp Lethality Bioassay: Brine shrimp bioassay method indicates cytotoxicity as well as a wide range of pharmacological activities e.g., anticancer, antiviral and pesticidal etc [10]. Brine shrimp eggs were hatched in simulated sea water to get nauplii. Test samples of different concentrations (400 µg/ml to 0.781 µg/ml) were prepared by dissolving in dimethylsulfoxide (DMSO). The nauplii were counted by visual inspection and were taken in vials containing 5 ml of simulated sea water. Then test samples were added to the pre-marked vials through micropipette and after 24 hours of incubation the survivors were counted. The LC_{50} (lethal concentration to half of the test organism) values of the test samples were calculated from the regression equation, prepared from the logarithm of sample concentration and percentage mortality of the shrimp nauplii.

Statistical Analysis: Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD. Correlation analysis of free radical scavenging activity versus total phenolics content and reducing power was carried out using the correlation and regression program.

RESULTS AND DISCUSSION: The present study was undertaken to evaluate the antioxidant activity of different organic soluble materials of a methanol extract of *S. nodiflora.* The results are shown in **table 1**. The total antioxidant capacity of the *S. nodiflora* extracts expressed as the mg of ascorbic acid was determined by phosphomolybdenum assay, where the highest value was found in dichloromethane soluble fraction followed by carbon tetrachloride and methanol extract as evident from 0.249 mg, 0.175 mg and 0.145 mg equivalents of ascorbic acid/100g of sample respectively.

The total phenolic content in the extractives was found in between 1.574 to 9.4136 mg of GAE/100gm of sample. The total phenolic content in MeOH extract was 6.327 mg of GAE/100gm of sample and its pet-ether, carbon tetrachloride, dichloromethane and aqueous soluble Kupchan fractions showed 1.574, 8.1790, 9.4136 and 5.895 mg of GAE/100gm of sample respectively. This indicated that the dichloromethane soluble fraction had the maximumt amount of phenolic compounds.

In the free radical scavenging (DPPH) assay, the IC_{50} values of the test fractions were found to be 10.52 - 31.25 µg/ml, where the lower IC_{50} value is indicative of higher free radical scavenging activity. The free radical scavenging activity of dichloromethane soluble fraction was higher among all the extractives, IC_{50} value was 10.52 µg / ml and its activity was found to be even better than the synthetic antioxidant, butylated hydroxyl toluene, here IC_{50} value was 27.5 µg/ml. The strongest activity of dichloromethane fraction is most probably due to its high phenolic content (9.4136 mg of GAE/g of sample).

TABLE 1: TOTAL ANTIOXIDANT CAPACITY, TOTAL PHENOLIC CONTENT AND FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT PARTITIONATES OF *S. NODIFLORA* AND STANDARDS*

Sample code	Sample description	Total phenolic content (mg of GAE/100 gm of dried extract)	Total antioxidant capacity (mg of ascorbic acid/100g of plant extract)	Free radical scavenging activity (IC_{50} µg/ml)
BHT	Butylated hydroxytoluene	-	-	27.5 ± 0.54
ASA	Ascorbic acid	-	-	5.8 ± 0.21
MESN	Methanol extract	6.327 ± 1.03	0.145 ± 0.22	17.79 ±2.08
PESN	Pet ether soluble fraction	1.574 ± 0.25	0.060 ± 0.75	31.25 ± 1.58
CTCSN	Carbon tetrachloride soluble fraction	8.1790 ± 1.78	0.175 ± 0.98	11.75 ± 0.79
DCMSN	Dichloromethane soluble fraction	9.4136 ± 0.89	0.249 ± 1.02	10.52 ± 0.28
AQSN	Aqueous soluble frafion of methanol extract	5.895 ± 0.57	0.140 ± 1.57	29.58 ± 1.29

*The average values of three calculations are presented as mean ± S.D. (standard deviation).

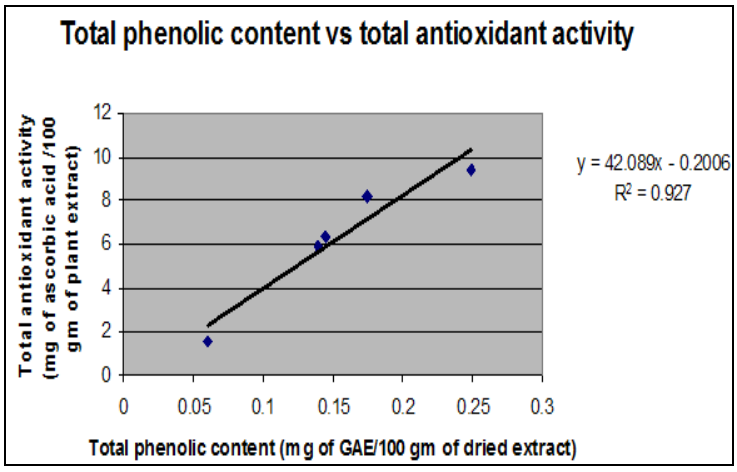

FIGURE 1. CORRELATION BETWEEN THE TOTAL PHENOLIC CONTENT AND TOTAL ANTIOXIDANT ACTIVITY

In the brine shrimp lethality bioassay, different mortality rate of the nauplii in all samples and no lethality/mortality of the control group suggest that the crude methanol extract of the whole plant and its pet-ether, carbon tetrachloride, dichloromethane and aqueous soluble fraction contain toxic activities. The results are shown in table 2. The LC_{50} values of the tested fractions were found in the range of 0.113 to 0.230 µg/ml where the lowest LC_{50} value (0.113 µg/ml) was revealed by the dichloromethane soluble fraction and the highest (0.23 µg/ml) from the methanol extract and pet-ether fraction, whereas the used standard Vincristine sulphate showed the LC_{50} value of 0.451 µg/ml.

TABLE 2. LC_{50} VALUES IN BRINE SHRIMP LETHALITY BIOASSAY OF DIFFERENT FRACTIONS OF *S. NODIFLORA* AND VINCRISTINE SULFATE

Samples	LD_{50} (µg/ml)
MESN	0.230
PESN	0.230
CTCSN	0.122
DCMSN	0.113
AQSN	0.119
Vincristine sulfate	0.451

CONCLUSION: From the above results, it may be concluded that, the whole plant of *S. nodiflora* has moderate antioxidant activity and strong cytotoxicity which warrant bioactivity guided isolation of the active compounds. The present findings from the preliminary toxicity studies of the extractives of *S. nodiflora* (L) support the insecticidal uses of the plant by the indigenous people.

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