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ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF *PARABAENA SAGITATTA* MIERS

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Abstract

A methanolextract of the leaves of *Parabaena sagitatta* Miers and its petroleum ether, carbon tetrachloride, dichloromethane, ethylacetate and aqueous soluble partitionates were evaluated for antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteau reagent and phosphomolybdenum total antioxidant assays by using butylated hydroxytolune (BHT) and ascorbic acid as standards. The dichloromethane soluble fraction demonstrated the presence of significant amount of phenolic compounds 61.06 ± 0.54 mg GAE/g of extract and also has moderate antioxidant activity IC_{50} 50.62 ± 0.25 µg/ml. A positive correlation (R^2), 0.969 was observed between total phenolic content and total antioxidant activity of *P. sagitatta*. The general toxicity was determined by brine shrimp lethality bioassay where the dichloromethane LC_{50} 0.978 µg/ml and carbon tetrachloride LC_{50} 1.45 µg/ml soluble partitionates demonstrated the presence of considerable bioactive principles.

Introduction

Parabaena sagittata Miers belonging to the family Menispermaceae is a lofty climber, indigenous to Chittagong, Bangladesh (Rashid and Rahman 2011). It is also found in northern part of Thailand and used by the hill tribes of this region for medical purposes. A decoction of stems and leaves affords a treatment for jaundice, indigestion and painful intestinal disturbances. All parts of the plant may be used as a febrifuge and tonic (Ruangrungsi *et al*. 1986). The leaf paste of *P. sagitatta* is boiled in coconut oil and is applied on incision (Dagar 1989).

Materials and Methods

The leaves of *P. sagitatta* were collected in mid 2010 from Dhaka University campus and a voucher specimen (DACB-35895) has been deposited in Bangladesh National Herbarium, Mirpur, Dhaka.

Plant materials were chopped, dried and powdered and about 600 g of the powdered material was soaked in 2.5 litres of methanol at 30°C for 7 days. The extract was filtered by using Whatman filter paper No. 1 and concentrated with a rotary evaporator. An aliquot of the concentrated methanol extract was partitioned by modified Kupchan method (Vanwagenen *et al*. 1993) and the resultant partitionates i.e. pet-ether (PSF), carbon tetrachloride (CSF), dichloromethane (DSF), ethyl acetate (EASF) and aqueous (ASF) soluble fractions were evaporated to dryness with a rotary evaporator. The residues were stored in a refrigerator until further studies.

The total phenolic contents of the extractives were determined with Folin-Ciocalteau reagent by using the method developed by Harbertson and Spayd (2006). To 0.50 ml of each sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteau reagent and 2.0 ml of sodium carbonate (7.5%, w/v) in water were added and incubated for 15 min at 45°C. The absorbance of all samples was measured at 765 nm with a visible spectrophotometer. The phenolic contents were expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of dry weight of extract.

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Following the method developed by Brand-Williams (1995) the antioxidant activity of the methanol extract and its sub-fractions was measured by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Brand-Williams *et al.* 1995). Then, 2.0 ml of the different concentrations (0.977 to 500 µg/ml) of the test samples were mixed with 3.0 ml of DPPH solution (20 µg/ml) in methanol. After 30 minutes of reaction period at room temperature in dark, the absorbance was measured at 517 nm as indicated earlier.

The IC_{50} values (concentration of samples required to scavenge 50% of free radicals) were calculated from the regression equation, developed by plotting concentration of the samples versus percentage inhibition of free radicals. Here, synthetic antioxidants, butylated hydroxy-toluene (BHT) and L-ascorbic acid were used as positive control.

The total antioxidant activity of the extract was evaluated by the phosphomolybdenum assay method (Prieto *et al.* 1999), the details of which has been published previously. The extract (2 mg/ml, 0.3 ml) was allowed to mix with 3.0 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 minutes. After cooling at room temperature, the absorbance of the solution was measured at 695 nm by using a UV-visible spectrophotometer against reagent blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.

Table 1. Total phenolic content, free radical scavenging activity, total antioxidant capacity and cyto-toxicity of *P. sagitatta.*

Sample	Total phenolic content (mg of GAE/g of dried extract)	Free radical scavenging activity (IC_{50} µg/ml)	Total antioxidant capacity (mg of ascorbic acid/100 g of plant extract)	Brine shrimp lethality bioassay LC_{50} (µg/ml)
Vincristine sulfate	-	-	-	0.451
BHT	-	27.50	-	-
Ascorbic acid	-	5.80	-	-
ME	18.26	95.65	0.613	2.936
PSF	12.85	145.60	0.114	4.011
CSF	47.64	55.25	1.565	1.452
DSF	61.06	50.62	2.0	0.978
EASF	22.73	60.45	0.756	1.703
ASF	15.66	120.15	0.205	3.931

The average values of three calculations are presented as mean ± SD (standard deviation); BHT = Butylatedhydroxytolune; ME = Methanol extract; PSF = Petrolium ether soluble fraction; CSF = Carbon tetrachloride soluble fraction; DSF = Dichloromethane soluble fraction; EASF = Ethyl acetate soluble fraction; ASF = Aqueous soluble fraction.

For screening of general toxic properties, which also indicates a range of bioactivities (anticancer, antiviral and pesticidal properties) (Meyer *et al.* 1982). Test samples of different concentrations (0.781 to 400 µg/ml) were prepared in dimethylsulfoxide (DMSO). Ten brine shrimp nauplii were taken in vials containing 5 ml of simulated sea water. Then test samples were added to the pre-marked vials with micropipette and after 24 h, the number of the survivors were counted and the LC_{50} was calculated from the regression equation, prepared from the logarithm of sample concentration versus percentage mortality of the shrimp nauplii.

Results and Discussion

The methanol crude extract of *P. sagitatta* as well as different Kupchan partitionates derived from it were subjected to assays for total phenolic content, free radical scavenging activity and preliminary cytotoxicity. The total phenolic content in the samples were found in the range of 12.85 - 61.06 mg of GAE/g of sample. The total phenolic content in crude extract was 18.26 mg of GAE/g of sample and as compared to dichloromethane, carbon tetrachloride, ethyl acetate, aqueous and pet-ether soluble fractions were 61.06, 47.64, 22.73, 15.66 and 12.85 mg of GAE/g extractives, respectively. The result indicated the highest amount of phenolic compounds in the dichloromethane soluble fraction.

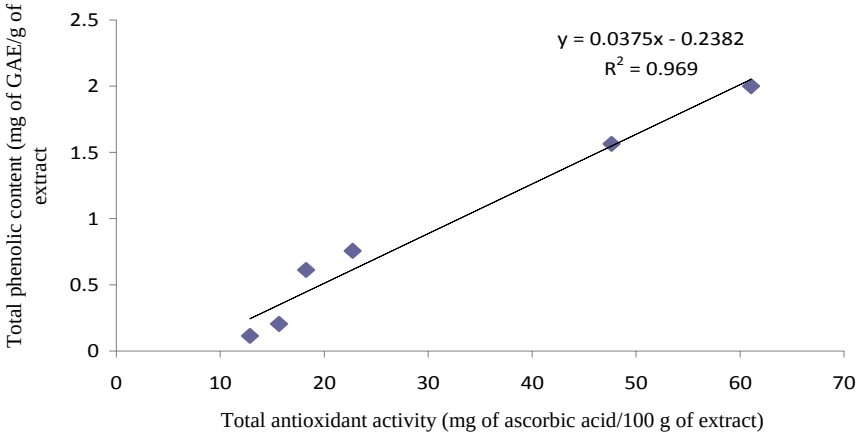

Fig.1. Correlation between total phenolic content and total antioxidant capacity of *P. sagitatta* extractives.

In the DPPH free radical scavenging assay, the dichloromethane soluble fraction revealed maximum free radical scavenging activity (IC_{50} = 50.62 µg/ml) when compared to butylated hydroxytoluene (IC_{50} = 27.5 µg/ml). This prominent free radical scavenging may be correlated to its high phenolic content (61.06 mg of GAE/g of sample) or due to synergistic activity of various chemical entities present in the extractive. A positive correlation was seen between total phenolic content and total antioxidant activity of *P. sagitatta* having correlation coefficient (R^2) values of 0.969.

In the brine shrimp lethality bioassay, the lowest LC_{50} (0.978 µg/ml) value was obtained with the dichloromethane soluble fraction, whereas Vincristine sulphate exhibited an LC_{50} value of 0.451 µg/ml.

It is clearly evident from the above findings that the leaves of *P. sagitatta* have significant antioxidant potential and cytotoxic properties. Therefore, the plant is a good candidate for further systematic chemical and biological studies to isolate the active principles.

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