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Antioxidant and Cytotoxic Activities of *Mussaenda macrophylla*

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Abstract

A methanol extract of the leaves of Mussaenda macrophylla and its petroleum ether, carbon tetrachloride, dichloromethane, ethyl acetate and aqueous soluble partitionates were evaluated for antioxidant activity by 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 (38.50 ± 0.64 mg GAE/g of extract) and also has moderate antioxidant activity (IC_{50} 42.95 ± 0.73 µg/ml). A positive correlation was seen between total phenolic content and total antioxidant activity of M. macrophylla having correlation coefficient (R^2) of 0.759. The general toxicity was determined by brine shrimp lethality bioassay where the carbon tetrachloride (LC_{50} 0.546 µg/ml) and dichloromethane (LC_{50} 0.611 µg/ml) soluble partitionates demonstrated the presence of considerable bioactive principles.

Key words: *Mussaenda macrophylla*, Antioxidant, DPPH, Cytotoxicity.

Introduction

Mussaenda macrophylla (Wall) belonging to the family Rubiaceae is a flowering shrub which is distributed in central and eastern Nepal to about 1800 m in moist places in association with herbs and other shrubs. It is also found to occur in northern India, southern China and Myanmar (Manandhar, 2002). Traditionaly the bark of this plant is used in Snake bite (Dictionary of Chinese traditional medicine, 1986). Previous studies with *M. macrophylla* revealed antibacterial, anticoagulant, anti-inflammatory and hepatoprotective activities (Dinda *et al*., 2009). The plant is also active against oral pathogen (Kim *et al.*, 1999). As a part of our continuing investigation of medicinal plants of Bangladesh (Kaisar *et al*., 2011; Kabir *et al*., 2010) the methanolic extract and fractions obtained from leaves of *M. macrophylla* growing in Bangladesh were investigated for the antioxidant activity in terms of total phenolic content and free radical scavenging activity as well as cytotoxicity by brine shrimp lethality bioassay for the first time.

Materials and Methods

Collection of plant materials and extraction: The leaves of *M. macrophylla* were collected in mid 2010 from Dhaka University campus and a voucher specimen (DACB - 35633) has been deposited in Bangladesh National Herbarium.

Collected plant materials were chopped, dried and powdered and about 600 gm of the powdered material was soaked in 2.5 litres of methanol at room temperature for 7 days. The extract was filtered by using Whatman filter paper number 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.

Total phenolic content: 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

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visible spectrophotometer. The phenolic contents were expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of dry weight of extract.

DPPH free radical scavenging assay: Following the method developed by Brand- Williums *et al*. (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 (500 µg/ml to 0.977 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 hydroxytoluene (BHT) and L-ascorbic acid were used as positive control.

Phosphomolybdenum antioxidant assay: 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.

Brine shrimp lethality bioassay: 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 (400 µg/ml to 0.781 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 hours, 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 methanolic crude extract of *M. macrophylla* 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 14.95 ± 0.56 to 38.50 ± 0.64 mg of GAE/g of sample. The total phenolic content in crude extract was 23.65±0.22 mg of GAE/g of sample and as compared to dichloromethane, carbon tetrachloride, aqueous, pet-ether and ethyl acetate soluble fractions were 38.50, 27.80, 25.25, 14.95 and 11.30 mg of GAE/g extractives, respectively. The result indicated the highest amount of phenolic compounds in the dichloromethane soluble fraction.

In the DPPH free radical scavenging assay, the dichloromethane soluble fraction revealed maximum free radical scavenging activity (IC_{50} = 42.95 ± 0.73 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 (38.50±0.64 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 *M. macrophylla* having correlation coefficient (R^2) values of 0.759 (Figure 1).

In the brine shrimp lethality bioassay, the lowest LC_{50} (0.546 µg/ml) value was obtained with the carbon tetrachloride 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 *M. macrophylla* have moderate antioxidant potential and significant cytotoxic properties. Therefore, the plant is a good candidate for further systematic chemical and biological studies to isolate the active principles.

Table 1. Total antioxidant capacity, total phenolic content and free radical scavenging activities and cytotoxicity of *M. macrophylla*.

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	-	27.5±0.54	-	-
Ascorbic acid	-	5.8±0.21	-	-
ME	23.65±0.22	220.85±0.21	0.423±0.61	0.897
PSF	14.95±0.56	270.32±0.45	0.288±0.52	1.49
CSF	27.80±0.45	95.95±0.84	1.201±0.25	0.546
DSF	38.50±0.64	42.95±0.73	1.276±0.45	0.611
EASF	11.30±0.25	140.45±0.26	0.211±0.11	2.461
ASF	25.25±0.22	125.95±0.15	0.459±0.15	2.01

The average values of three calculations are presented as mean ± S.D. (standard); BHT= Butylated hydroxytolune; ME= Methanol extract; PSF= Pet ether soluble fraction; CSF= Carbon tetrachloride soluble fraction; DSF= Dichloromethane soluble fraction; EASF= Ethyl acetate soluble fraction; ASF= Aqueous soluble fraction.

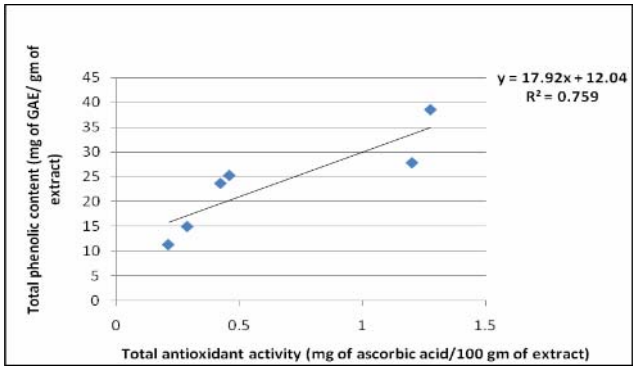

Figure 1. Correlation between total phenolic content and total antioxidant capacity of *M. macrophylla* extractives.

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