

Evaluation of Antioxidant Activity and Cytotoxic Property of Methanolic Extract of *Flemingia macrophylla* (Willd.)

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Abstract

Flemingia macrophylla locally known as 'Charchara' in Bangladesh is a medicinal herb. Being a member of the Fabaceae family it claims a number of medicinal uses including hypoglycemic activity, neuroprotective effect and gynecological remedies. Methanolic extract of *F. macrophylla* was investigated to evaluate its antioxidant and general toxic properties in this study. Antioxidant potential was evaluated using total antioxidant capacity, total phenol contents, total flavonoid contents and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays. Both leaf and stem extracts of *F. macrophylla* were found to possess significant amount of phenolics and flavonoids, expressed as gallic acid equivalents (GAE) and quercetin equivalent (QE), respectively. Interestingly total phenol content was equal for both leaf and stem extract and it was 43.8 mg GAE/g. Total flavonoid content was found to be 64.4 and 39.1 mg QE/g for leaf and stem extract, respectively. The total antioxidant capacity was expressed as ascorbic acid equivalents (AAE) and for leaf it was 5.067 mg/gm AAE and for stem 0.8167 mg/gm AAE. DPPH scavenging activity was determined by comparing with ascorbic acid. The IC₅₀ values were 19.95, 125.89 and 707.94 µg/ml for ascorbic acid, leaf and stem extract, respectively. The results of the present study on methanolic extracts of *F. macrophylla* revealed the presence of moderate antioxidant activity and extract of leaves produced better results than stem of the plant. In brine shrimp lethality bioassay, methanolic extract of *F. macrophylla* exhibited insignificant cytotoxicity.

Key words: *Flemingia macrophylla*, Antioxidant, DPPH Scavenging activity, Cytotoxicity.

Introduction

Plants have been the basis of many traditional medicines throughout the world for thousands of years and continue to provide new remedies (Samuelsson, 2004). Many drugs used in contemporary medicine have been derived from plants and were originally discovered through the traditional use by the indigenous people. This study is based on ethnobotanical knowledge of the plant *Flemingia macrophylla*. It is a woody, deep-rooting, tussock-forming shrub, 1-4 m tall. Different parts of *Flemingia* species have been reported useful traditionally for treating different kinds of diseases, like roots for rheumatism, arthropathy, melangia, chronic nephritis, etc. (Lie *et al.*, 2008); stems in rheumatism, inflammation, etc. (Hsieh *et al.*, 2010). *F. macrophylla* has also been found to possess hepatoprotective and neuroprotective activities (Hsieh *et al.*, 2011; Young *et al.*, 2005).

Antioxidants from the natural source are very much promising in the present natural product studies due to their better efficacy with less adverse effects. Antioxidants protects cell damage significantly scavenging the free radicals and reactive oxygen species developed in various diseased conditions like cancer, hepatic failure, diabetes mellitus, arteriosclerosis, inflammation, renal failure, atherosclerosis, neurodegenerative disorders, etc. (Bulkley, 1983; Dormandy, 1983; Niki, 1995; Frei, 1999). Therefore, in our present study, it was aimed to investigate and compare the antioxidant activity of methanolic extracts of leaf and stem of *F. macrophylla*. Moreover, for screening of general toxicity and cytotoxic properties, the brine shrimp lethality assay was also conducted.

Materials and Methods

Plant material: *Flemingia macrophylla* was collected from Chandra, Gazipur and the botanical identity was authenticated by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. The voucher specimens (DACB-35624) of the plant have been deposited in the herbarium for future reference.

Drugs and chemicals: 1,1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin and gallic acid were obtained from Sigma Chemical Co (MO, USA). Folin-ciocalteu reagent (FCR) and Griess reagent were purchased from Merck, Germany. All other chemicals and reagents were of analytical grade.

Extraction: The leaves and stems of the plant were collected in fresh condition. They were sun-dried first and then dried in an oven at reduced temperature (<50 °C) to facilitate grinding. 350 gm powdered materials for each part were submerged separately in 1500 ml methanol into two air-tight flat bottomed containers for seven days, with occasional shaking and stirring. The extracts were then filtered and dried on an electrical water bath at 50 °C.

Phytochemical screening of crude plant extract: In phytochemical screening the methanolic extracts were qualitatively checked for various components using the following reagents and chemicals – Dragendorff's reagent for alkaloids, Mg and HCl for flavonoids, Ferric chloride and Potassium dichromate solutions for tannins, Libermann-Burchard reagent for saponins and Molisch's test for carbohydrates and general test for glucosides. Components were identified by observing the Characteristic color changes (Trease and Evans, 1983).

Determination of total phenols: Total phenols were determined by Folin-Ciocalteu reagent (Folin and Ciocalteu, 1927). 1.0 ml of each plant extract (10 µg/µl) or standard of different concentrations (250, 200, 150, 100 and 50 µg/ml) were taken in different test tubes. 5 ml of Folin-Ciocalteu (diluted 10 fold) and 4 ml of Sodium carbonate reagent was added to the test tubes. The test tubes were incubated for 30 minutes at 20°C to complete the reaction. The absorbance of the solutions was measured at 765 nm using a UV-Vis spectrophotometer against distilled water as blank. Total phenolic compounds in plant extracts were expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound. The result was calculated from the

regression equation of the calibration curve ($y=0.009x + 0.058$; $R^2=0.997$).

Determination of flavonoid content: Aluminum chloride colorimetric method was used for flavonoids determination (Chang *et al.*, 2002). 1 ml of sample was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water. It was kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solution at concentrations 12.5 to 100 µg/ml in methanol.

Determination of total antioxidant capacity: The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto *et al.*, 1999). The antioxidant capacity was expressed as ascorbic acid equivalent (AAE). The plant extract (0.3 ml) was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95°C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against an appropriate blank. Total antioxidant capacity of extract was measured from the calibration curve constructed by using ascorbic acid standard solutions.

DPPH scavenging activity: DPPH free radical scavenging activity of the extracts was measured by the method developed by Manzorro *et al.* (1998). 0.2 ml of sample (extract or standard) of each concentration (500, 200, 100, 50, 10, 5 µg/ml) were mixed with 2 ml of DPPH solution (0.5 mM). After 30 minutes of reaction at room temperature in dark place, the absorbance was measured at 517 nm. IC₅₀ values (concentration of samples required to scavenge 50% of free radicals) were calculated from the regression equation, developed by plotting concentration of samples versus percentage inhibition of free radicals. Ascorbic acid was used as positive control.

Brine shrimp lethality bioassay: This technique was applied for determination of general toxic property of the plant extract (Meyer *et al.*, 1982 and McLaughlin *et al.*, 1998). Test samples of different concentrations (500 µg/ml to 1 µg/ml) were prepared in dimethylsulfoxide (DMSO). Ten brine shrimp nauplii were taken in vials

containing 5 ml of simulated sea water. Then samples were added to pre-marked vials with micropipette. After 24 hours and the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. Vincristine sulphate was used as positive control in this study.

Results and Discussion

Since the chemical constituents present in a plant are directly responsible for its therapeutic and other pharmacological properties, the constituents of the plant which are detected during this investigation should have some direct relationship with local medicinal uses. The results of various qualitative chemical tests for the detection of chemical constituents of *F. macrophylla* are shown in the Table 1.

Table 1. Results of phytochemical screening of methanol extract of different parts of *F. macrophylla*

Plant parts	Carbohydrate	Glycosides	Glucosides	Anthraquinone glycoside	Alkaloids	Saponins	Flavonoids	Steroids	Tannins
Stem and Leaf	+	+	-	+	±	-	+	+	+

‘+’ Indicates Positive Reaction, ‘-’ Indicates No Reaction, ‘±’ Indicates Presence or Absence could not be ascertained

Table 2. Antioxidant capacity of different parts of *F. macrophylla*.

Sample	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)	DPPH free radical scavenging activity (IC ₅₀ µg/ml)	Total antioxidant capacity (mg AAE/g)
Ascorbic Acid			19.95 ± 0.22	
Leaf	43.8 ± 0.22	64.4 ± 0.56	125.89 ± 0.45	5.067 ± 0.31
Stem	43.8 ± 0.13	39.1 ± 0.29	707.94 ± 0.21	0.8167 ± 0.26

The content of total phenolics in the methanolic plant extracts was determined using the Folin-Ciocalteu assay. The content of phenolics was found to be 43.8 mg GAE/g in each extracts (Table 2). The antioxidant activity of the phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

Flavonoid content was calculated from the regression equation of the calibration curve ($y = 0.005x - 0.005$) and is expressed as quercetin equivalents (QE). The flavonoid content was determined as 64.4 mg QE/g for leaf extract and 39.1 mg QE/g for stem extract (Table 2). Flavonoids are polyphenolic compounds that are ubiquitous in nature and are mainly categorized as flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidines and chalcones according to their chemical structures.

The total antioxidant capacities of the methanolic extracts of the selected plant were determined from the calibration curve established by ascorbic acid at 695 nm. The regression line was $y=0.006x+0.0101$ and $R^2=0.991$. The ascorbic acid equivalent (AAE) for leaf and stem extract were 5.067 and 0.8167 mg/g, respectively.

In DPPH scavenging assay, IC₅₀ values of the methanolic extracts of *F. macrophylla* were found to be 125.89 and 707.94 µg/ml for leaf and stem, respectively. IC₅₀ value for ascorbic acid was 19.95 µg/ml. Fig 1 shows the amount of extract needed for 50% inhibition (IC₅₀).

Yet now most of the reliable work has been performed for the ‘aqueous’ extract of *F. macrophylla*. So this work is a new conjunction for this research. DPPH reading of the plant extract is not very close to the Ascorbic acid. It is because the plant extract is in crude form and it is quite possible to be a potent antioxidant

after bioactive compound isolation. In similar work with the aqueous extract of *F. macrophylla*, IC_{50} value was found 113 $\mu\text{g/ml}$ (Syiem *et al.*, 2009) which is close to this study result for the same (IC_{50} =125 $\mu\text{g/ml}$ for leaf extract). In the same experiment conducted by Syiem *et al.* (2009), total phenol content was found as 200 and 12.7 mg GAE/g for the methanolic and aqueous extract, respectively whereas, in our study, the value for total phenol content was found 43.8 mg GAE/g for both of the methanolic extracts of leaf and stem of *F. macrophylla*. The reasons for variation in findings might be due to nature of soil,

condition of growth of plants method variation, variation in chemical grade and UV detection error. Total flavonoid content and total antioxidant reading also showed satisfactory result. It is clear from all of the experiments that the 'Leaf' extract has greater antioxidant property than the 'Stem' extract although both of them exhibited moderate antioxidant activity. Moreover, the positive result found for glycoside, anthraquinone glycoside, flavonoid and tannin in phytochemical screening also gave indication for antioxidant property.

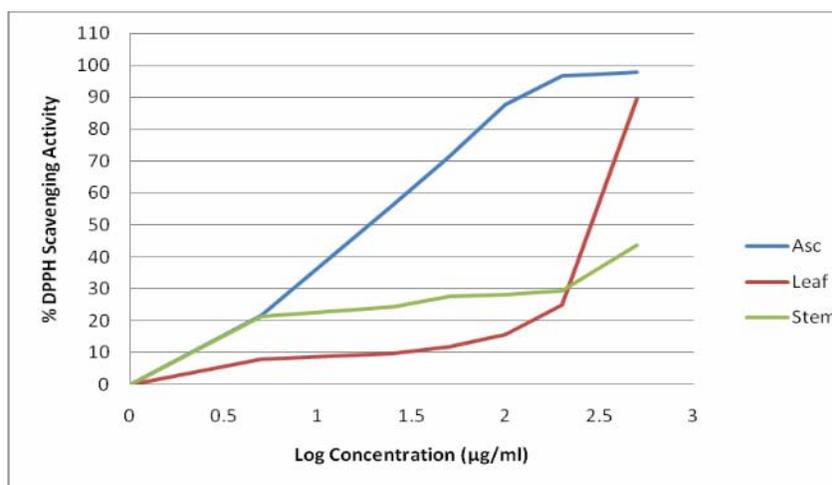


Figure 1. comparative DPPH scavenging activity.

In brine shrimp lethality bioassay, after 24 hours all of the samples showed insignificant lethality. Median lethal concentration or LC_{50} values were found 174582.2 $\mu\text{g/ml}$ for stem extract and 889.962 $\mu\text{g/ml}$ for leaf extract, whereas the reference standard vincristine sulphate exhibited LC_{50} values of 0.45 $\mu\text{g/ml}$. It is clearly evident from the above findings that the leaves and stem extracts of *F. macrophylla* have moderate antioxidant activity but almost no cytotoxic property. Future endeavors to isolate the active principles from this plant can assist to open up exciting new therapeutic avenues by finding fundamental medicinal potentiality.

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