WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 5, 334-344. Research Article ISSN 2277– 7105

TOTAL PHENOL, TOTAL FLAVONOID CONTENT AND ANTIOXIDANT POTENTIAL OF METHANOL EXTRACT OF *BOEHMERIA PLATYPHYLLA* D DON LEAVES.

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Article Received on
08 March 2016,
Revised on 29 March 2016,
Accepted on 20 April 2016
DOI: 10.20959/wjpr20165-6159

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ABSTRACT

Background: *Boehmeria platyphylla* leaves are deliberated as worthy traditional medicine. To give a scientific basis for traditional usage of this medicinal plant, the leaf extract were appreciated for its antioxidant activity. **Methods:** In this study, *in vitro* antioxidant activities of the leaf extract of *B. platyphylla* were determined by total antioxidant, DPPH, Reducing power capacity, total phenolic content and total flavonoid content. **Result:** The extract showed a dose dependent radical scavenging effect in DPPH assay. IC_{50} for free radicals achieved by the extract is 144.72 µg/ml. The extract showed significant reducing power activity as compared to ascorbic acid and proportionally increased with the increasing concentration of the extract. Increase in absorbance of the reaction mixture indicates the increase in the reducing power of the extract. Phenol content was 18.44 ± 0.45 mg gallic acid/g and flavonoid content was 21.12 ± 0.23 mg quercetin/g. **Conclusion:** Our current results emerged that *B. platyphylla* act as an antioxidant agent due to its free radical scavenging and cytoprotective activity. So, the plant may be further pursued to find out for its pharmacological active natural products.

KEYWORDS: *Boehmeria platyphylla*, phenol, flavonoid, antioxidant, DPPH.

1. INTRODUCTION

An expanding human population, global environment change and the change of terrestrial food resources for energy needs in recent times have elevated serious global food security concerns **(Kumar et al., 2011)**. There has been a quest to explore and use foods from diverse sources to enhance and supplement the nutritional quality of human foods. Non timber forest products used in diet have recently gotten an increasing interest as they constitute a developing source of food. Organic and natural compounds have been reported to have own antioxidant properties, bioactivities and applications of preparations isolated from herb, flower, vegetable, which most frequently include berries, fruits, fresh fruit and vegetables, medicinal, aromatic plants, herbs and other botanicals have been well documented **(Biapa et al., 2011; Choumessi et al., 2012; Dimo et al., 2001)**. Polyphenols are bioactive compounds extensively spread in plants and they are generally} also significant constituents of the human diet **(Pauline et al., 2013)**. Plants are considered as the key sources of antioxidants, which constitute a rich diversity of substances such as flavonoids (anthocyanins, flavonols, flavones) as well as some classes of non-flavonoids (phenolic stomach acids, lignins, stilbenes, terpenoids and many others). These compounds vary in structure, the quantity of phenolic hydroxyl groups and the location, leading to variance in their antioxidative and biological potential **(Erkan et al., 2011)**. Some culinary herbs and spices have been proven to be more effective antioxidants than common food additives (butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate) and vitamins (ascorbic acid) **(Suhaj, 2006)**. Therefore herbs and spices rich in anti-oxidants and other phyto-compounds are able to prevent oxidative stress and its related disorders such as persistent diseases **(Soory, 2009; Tapsell et al., 2006)**.

Hence, investigations in naturally occurring antioxidants has considerably increased and natural products have regained prominence in the recent past with increasing understanding of their biological significance such as antioxidant, radical scavenging activities and increasing recognition of the origin and function of their structural diversity **(Deng et al., 2006; Gul et al., 2011; Hogan et al., 2010; Zhang et al., 2007)**. It then becomes necessary to search new source for noble antioxidants, especially those that would be safe and cheap and thus easily affordable by all population.

Boehmeria platyphylla is a monoecious or dioecious, 1-1.5 (-3) m tall, shrub with 4-angled, glabrescent twigs. Leaves mostly opposite, with 2.5-20 cm long petiole; lamina 3-costate,

broadly ovate to orbiculate, 6-22 cm long, 5-15 cm broad, sparsely appressed hairy, scabrous, dentate, somewhat cuneate, truncate or subcordate at the base, acuminate; stipules triangular-lanceolate, 8-12 mm long. Cymose clusters of flowers arranged on axillary, drooping, up to 30 cm long spikes. Flowers white, tetramerous; bracts lanceolate, 3-4 mm long. Sepals c. 1 mm long, pubescent, acute. Stamens exserted. Style long exserted. Achenes pale brown, c.l mm long, beaked, glossy. *B. platyphylla* is a species of plant in the Urticaceae family. The Urticaceae are subject to many bacterial, viral, fungal, and nematode parasite diseases [The American Phytopathological Society. Common Names of Plant Diseases: Diseases of Foliage Plants (House Plants): Urticaceae". 26 March 1993. Archived from the original on 30 November 2011] **(Chase, 1983; Nguyen, Mathur, & Neergaard, 1973)**. Leaves extract of *B. platyphylla* has brine shrimp lethality bioassay, thrombolytic and antibacterial activities **(Uddin MS, 2016)**.

The aim of the present study was to identify the antioxidant potential of methanol extract of *B. platyphylla* leaves. The extract was examined for DPPH free radical scavenging activity, reducing power capacity and for phenol and flavonoid content.

2. MATERIAL AND METHOD

2.1 Plant material

Fresh leaves of *B. platyphylla* were collected from Bandarban, Chittagong, Bangladesh in the month of March 2015. It was authenticated by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

2.2 Preparation of Extract

The leaves were dried for a period of 10 days under shade and ground. The ground leaves (450 gm) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then the whole mixture was filtered and the filtrate thus obtained was concentrated using a water bath to get a viscous mass. The viscous mass was kept at room temperature under a ceiling fan to get a dried extract (yield value, 5.3%). The extract prepared was for pharmacological screening.

2.3 Chemicals and equipment

All other chemicals and reagents were of analytical grade. Methanol purchased from Merck (India). Gallic acid, Folin-Ciocalteau reagent, trichloroacetic acid (TCA) was purchased from Sigma Chemicals Co. (P.O. Box 14508, St. Louis, MO 63178 USA). 1, 1-diphenyl-2-

picrylhydrazyl (DPPH), aluminium chloride was purchased from Fluka (Fluka chemie GmbH, CH-9471 Buchs). Ascorbic acid, Quercetin was purchased from BDH Chemicals (BDH Chemicals Ltd. Poole, England). Ferric chloride, potassium ferricyanide, sodium hydroxide and sodium nitrite were purchased from Riedel-De Haen Ag, Seelze-Hannover, Germany. Shimadzu Biospec 1601 UV visible spectrophotometer (Shimadzu, Japan) was used to measure the absorbance.

2.4 *In vitro* Antioxidant Activity

2.4.1 DPPH free radical scavenging activity

DPPH scavenging activity was carried out using the method of **Braca et al., 2001**. Different concentrations (400, 200, 100, 50, 25 and 12.5 µg/mL) of *B. platyphylla* extract were dissolved in methanol and placed in different test tubes, and 3 mL of a 0.004% w/v methanol solution of DPPH was added to each test tube. Absorbance at 517 nm was determined after 30 min against a blank, and the percent inhibition activity was calculated from $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. Ascorbic acid was used as a reference standard and dissolved in methanol to make the stock solution with the same concentration. The control sample was prepared containing the same volume without any extract or reference drug. Methanol served as a blank. The inhibition curves were prepared and the half maximal inhibitory concentration (IC_{50}) values were calculated using linear regression analysis.

2.4.2 Reducing power capacity

The reducing power of the extract was evaluated by the established method described by Oyaizu **(Oyaizu, 1986)** with slight modification. Different concentrations of leaf extract of *B. platyphylla* (125, 250, 500, and 1000 µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH = 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1% w/v). The mixture was incubated at 50°C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid solution was added to each tube and the mixture was centrifuged at 3000 rpm for 10 min. Subsequently, 5 mL of the upper layer solution was mixed with 5 mL of distilled water and 1 mL of ferric chloride solution (0.1% w/v), and the absorbance was measured at 700 nm. The reducing power of the extract was linearly proportional to the concentration of the sample. Ascorbic acid was taken as a reference standard. Phosphate buffer (pH 6.6) was used as a blank solution.

2.4.3 Determination of total phenolic content

Total phenolic content of the extract was evaluated with Folin-Ciocalteu method **(Uddin et al., 2015)**. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, 0.5mL aliquots of 12.5, 25, 50, 100, 200 and 400 µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin-Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. After incubation at 25°C for 30 min, the quantitative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer 1650. The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extract as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of extract.

2.4.4 Determination of total flavonoid content

Total flavonoid content of ethanol extract was evaluated with method of Jiao **(Jiao & Wang, 2000)**. One ml of *B. platyphylla* extract or standard of different concentrations was taken in a test tube and 3 ml of methanol was added. Then 200 µl of 10% aluminium chloride solution was added into the same test tube followed by the addition of 200 µl of 1M potassium acetate. Finally, 5.6 ml of distilled water was mixed with the reaction mixture. The reaction mixture was then incubated for 30 min at room temperature to complete the reaction. Then the absorbance of the solution was measured at 415 nm using a spectra photometer against blank. Methanol served as blank. The Total content of flavonoid compounds in *B. platyphylla* extract was expressed in mg/g quercetin equivalent (QE).

2.5 STATISTICAL ANALYSIS

All results are expressed as mean ± standard error of the mean (SEM). The results were statistically analyzed using repeated measures analysis of variance with Dunnett's multiple comparison when compared against negative control in all *in vivo* model of Sedative and Anxiolytic activities. $P<0.05$, $P<0.01$ and $P<0.001$ were considered as statistically significant. Statistical programs used were SPSS (Statistical Package for Social Science, version 22.0, IBM Corporation, NY). GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) was used for graphical presentation.

3 RESULTS

3.1. *In Vitro* Antioxidant Activity

3.1.1. DPPH radical scavenging activity

Results for the free radical scavenging activity of methanol extract of *B. platyphylla* are shown in **Figure 1**. The extract showed a dose dependent radical scavenging effect in DPPH assay. The half inhibition concentration (IC50) for free radicals achieved by the extract is 144.72 µg/ml which is statistically significant compared to that (IC50 8 µg/ml) of reference antioxidative agent ascorbic acid.

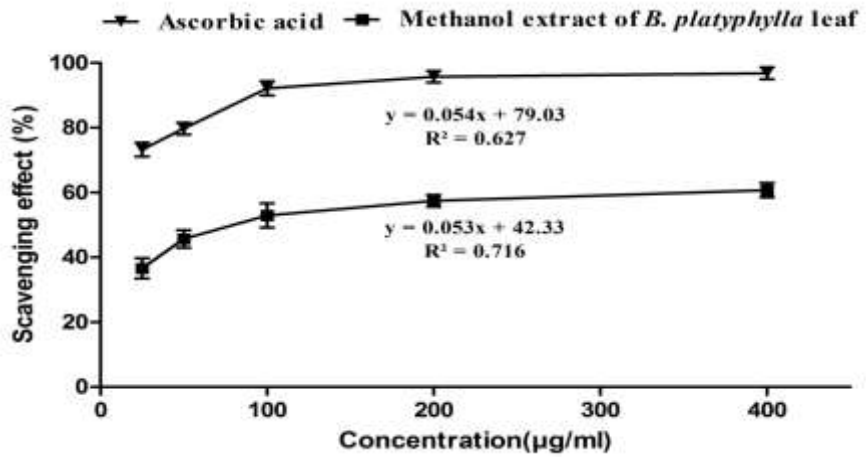

Figure 1. DPPH radical scavenging activity of *B. platyphylla* leaves.

3.1.2. Reducing power capacity

The extract showed significant reducing power activities as compared to ascorbic acid and proportionally increased with the increasing concentration of the extract, which is shown in **Figure 2**. Increase in absorbance of the reaction mixture indicates the increase in the reducing power of the sample.

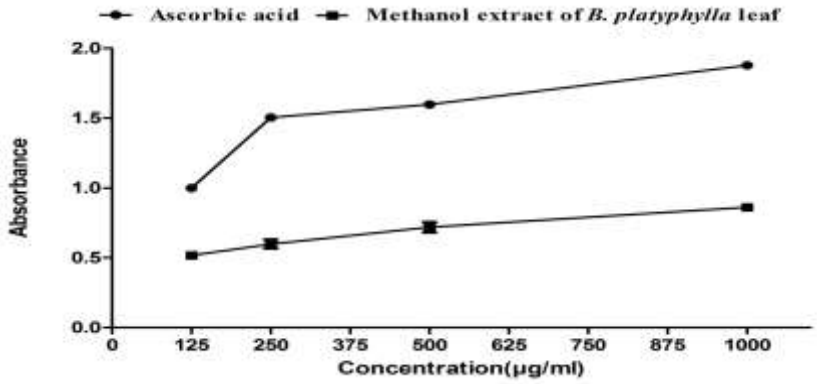

Figure 2. Reducing capacity of the methanol extract of *B. platyphylla* leaf.

3.1.3. Quantitative determination of phytochemical contents

Data for total phenolic and total flavonoid content has been summarized in **Table 1**. Data shows that moderate amount of total phenols and total flavonoids are present in the extract and the amounts are 18.44 ± 0.45 mg Gallic acid /g dry wt and 21.12 ± 0.23 mg Quercetin/g dry wt of dried extract respectively.

Table 1. Phytochemicals of methanol extract of *B. platyphylla*.

Phytochemicals (mg/gm)	*B. platyphylla* (methanol)
Total Phenol (mg Gallic acid /g)	18.44 ± 0.45
Total Flavonoid (mg Quercetin/g)	21.12 ± 0.23

Values are the mean of triplicate experiments and represented as mean ± SEM (n=3).

4 DISCUSSIONS

From best of our knowledge, present study was the first make an effort to evaluate the ability of the leaves extract of *B. platyphylla* to act as antioxidant agents. The most natural antioxidants are multifunctional. Consequently, a trusted antioxidant analysis process requires different antioxidant activity assessments to account various mechanisms of antioxidant action. in this study, several techniques have been used to determine the in vitro antioxidant activity to to let quick screening of substances.

DPPH radical scavenging model is widely used method to evaluate antioxidant activity of natural compound and plant extracts. The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is due to the hydrogen donating ability **(Barreira et al., 2008)**. The experimental data revealed that methanol extracts of leaves have the effects of scavenging free radicals and a dose dependent relationship in the DPPH radical scavenging activity. The involvement of free radicals, especially their increased production leads to the development of cardiovascular diseases and cancer. Thus, the consumption of *B. platyphylla* leaves can be beneficial in preventing oxidative stress related numerous chronic diseases.

The reducing power of MEBP was determined by direct electron donation in the reduction of ferri cyanide $[Fe(CN)^6]^{3-}$ to ferro cyanide $[Fe(CN)^6]^{4-}$. The product was visualized by addition of free Fe^{3+} ions after the reduction reaction, by forming the intense Prussian blue colour complex, $(Fe^{3+})_4[Fe^{2+}(CN^-)^6]_3$ and quantified by absorbance measurement at 700 nm **(Saha et al., 2013)**. The presence of reductants (i.e. antioxidants) in *B. platyphylla* leaves cause the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form which was monitored by measuring the formation of Perl's Prussian blue at 700 nm. Figure 2 shows the

reductive capabilities of MEBP compared to ascorbic acid. Therefore, like the DPPH radical scavenging activity, the observed reducing power of leaves and stem bark was in agreement with the chemical constituents in the extracts of *B. platyphylla* leaves.

Polyphenols were found in the extracts of leaves. The obtained results for DPPH are in agreement with the phenol contents determined for each sample. Plant polyphenols are produced from phenylalanine or from its precursor shikmic acid. These phenolics are important dietary antioxidants because they have ideal structural chemistry for free radical scavenging activities, and have been shown to be more effective antioxidants in vitro than vitamins E and C on a molar basis **(Ribeiro et al., 2008)**. Polyphenols exhibit a wide range of biological effects such as protection of LDL oxidation in vivo with significant consequences in atherosclerosis and also protect DNA from oxidative damage with important consequences in the age-related development of some cancers **(Reddy et al., 2012)**. Our findings suggested that leaves of *B. platyphylla* rich in phenolic and flavonoid contents which are the major contributor to scavenge the free radicals in oxidation pathways.

The results obtained from correlation between polyphenols (phenol and flavonoid) and DPPH scavenging suggested that phenolic compounds are dominant contributors to the antioxidant activity of the extract.

5 CONCLUSIONS

The present study indicated that *B. platyphylla* contains considerable amount of total polyphenols and flavonoids and exhibited good antioxidant activity by effectively scavenging various free radicals. The antioxidant and biological activities might be due to the synergistic actions of bioactive compounds present in them. However, it is still unclear which components are playing vital roles for this activity. Therefore, further studies are still needed to elucidate mechanistic way how the plant contributes to this property.

Competing interests

The authors declare that they have no competing interests.

ACKNOWLEDGMENT

The authors are grateful to the authority of International Islamic University Chittagong, Bangladesh, for providing the facilities to conduct this research work. The authors would like to thank, the Taxonomist and Professor, Dr. Shaikh Bokhtear Uddin, Department of Botany,

University of Chittagong, for identifying the plants. Authors would also like to thank Mr. Md. Mominur Rahman, Assistant professor, International Islamic University Chittagong, Bangladesh for his supervision in the experiments. The authors are also thankful to GUSTO (A research group) for the financial support and to all members of GUSTO (A research group) for their kind help in the experiments.

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