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Evaluation of Free Radical Scavenging and Polyphenolic Contents of Bark of *Pterygota alata* Roxb

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Abstract

Pterygota alata is a large deciduous tree of Malvaceae family. The present study was designed to evaluate *in vitro* antioxidant activity of stem bark of the plant. Ferric reducing power (FRP) test, 1, 1- diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging test and total antioxidant capacity (TAC) assay were used to detect the antioxidant activity. Total phenolic and flavonoid contents were also determined as they are well known phytochemicals with antioxidant property. The bark of the plant showed efficient reducing power as well as free radical scavenging property (IC_{50} values 52.25 - 172.05). The bark also found rich in total phenolic and flavonoid content. The highest amount of total phenolic content was found in chloroform soluble fraction (29.898 µg/mL) followed by ethyl acetate soluble fraction (15.88 µg/mL). The highest content of total flavonoid also detected in chloroform soluble fraction (107.56 µg/mL) but followed by crude ethanol extract (98.66 µg/mL). Overall, the bark of the plant possesses significant antioxidant activity, therefore can be used as a good natural source of antioxidant.

*Keywords***:** Free radical; Reactive oxygen species; DPPH; Ferric reducing power; Polyphenols.

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1. Introduction

Free radicals play important roles in a number of biological processes such as energy production, phagocytosis, regulation of cell growth and synthesis of biologically important compounds [1]. However, excessive amount of these free radicals can participate in unwanted side reactions, resulting in cell damage and contribute to diseases such as cancer, stroke, myocardial infarction and diabetes [2]. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur [3, 4]. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called free radical or reactive oxygen species (ROS) such as the hydroxyl radical, the superoxide anion radical,

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hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides [3, 4]. ROS are capable to react with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage.

The human body naturally produces antioxidants that are capable to mop up free radicals by neutralizing them, as a result prevent free radicals or ROS induced cell damage. But the process is effective in case of overwhelming production of free radicals [5, 6]. Antioxidants in food have received a great amount of attention as they possess primary preventive ingredients against various diseases [7, 8]. Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters are efficient against ROS, but concurrently cause or prompt negative health effects like liver damage and mutagenesis. As a result, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants [9-12]. Hence, several attempts to replace synthetic antioxidants with natural anti-oxidants have been developed. Recently, a number of plant products including phenolic and flavonoid contents as well as various crude extracts of plants were reported for antioxidant actions [13-17].

P. alata (family Malvaceae) is widely distributed in South Asia and Myanmar. Seeds of this plant have narcotic properties and used as substitute for opium. In India, seeds are eaten, and plant used medicinally [18, 19]. However, there has been little rigorous scientific study on this indigenous plant, and there is no scientific information on antioxidant properties of bark of this plant. In continuation the searching of new safe and cheap antioxidant preparation, the present study was designed to evaluate the antioxidant potential of extracts from barks of *P. alata*.

2. Materials and Methods

2.1. *Plant material*

P. alata is commonly known as Buddha narikal (English name: Buddha coconut) tree, was collected from University of Rajshahi, Rajshahi, Bangladesh in the month of February, 2013. The plant was identified by Dr AHM Mahabubur Rahman, Associate Professor of Department of Botany, University of Rajshahi and a voucher specimen has been deposited in the departmental herbarium with accession no. PH-116. The collected plant parts were dried for one week and pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place.

2.2. *Extract preparation*

Approximately 380 g of powdered bark was placed separately in a clean, flat-bottomed glass container and soaked in ethanol. The container with its contents was sealed and kept

for 7 days accompanied by occasional shaking and stirring. The entire mixture then underwent a coarse filtration by a piece of clean white cotton. The extract was re-filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). After filtration, the filtrate was evaporated to dryness at 50°C under reduced pressure using a rotary evaporator to obtain the ethanolic crude extract (9.5 g for leaves and 8.5 g for bark). The crude ethanolic extract (CEE) was further partitioned with n-hexane, chloroform, ethyl acetate and water. The resultant partitionates i.e., hexane (HF), chloroform (CHF), ethyl acetate (EAF) and water (AQF) soluble fractions were used for the biological screenings.

2.3. *Chemicals*

1,1-diphenyl-2-picrylhydrazyl (DPPH) and gallic acid were purchased from Sigma-Aldrich USA. Folin-Ciocalteu was obtained from Merck (Damstadt, Germany). Potassium ferricyanide, potassium acetate, prepared phosphate buffer (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 per L), catechin (CA), ferrous ammonium sulphate, ascorbic acid (AA), $AlCl_3$, trichloro acetic acid (TCA), sodium phosphate, ammonium molybdate, tannic acid, quercetin (QU), EDTA, acetyl acetone and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the chemicals used in the study were of analytical grade.

2.4. *Phytochemical screening of crude ethanolic extract*

Small amount of crude ethanolic extract of *P. alata* bark was dissolved in a suitable solvent and applied as small spot on the activated thin layer chromatography (TLC) plate. The plates was run with the solvent systems: 100% chloroform, 70% chloroform + 30% n-hexane, 50% chloroform + 50% n-hexane, 30% chloroform + 30% n-hexane + 40% methanol, and visualized with various spray reagents (vanillin-sulfuric acid spray, ceric sulfate-sulfuric acid spray, Dragendorff's spray, aluminium chloride spray, 4-aminoantipyrine/potassium hexacyanferrate (III) spray, p-anisaldehyde – sulfuric acid spray, ethanolamine diphenylborate, chloranil reagent spray) to determine the presence of various classes of active chemical constituents such as alkaloids, glycosides, steroids, flavonoids, saponins, tannins and terpenes etc. using standard procedures [20].

2.5. *Antioxidant assay*

2.5.1. *DPPH free radical scavenging assay*

The free radical scavenging activity of the extract as well as their various fractions was evaluated according to Braca *et al.* [21, 22]. Briefly, sample solution with different concentrations (ranging from 0 to 200 μg/mL) was mixed with 0.3% of DPPH methanol solution. The reaction mixtures were incubated at room temperature and allowed to react for 30 minutes in the dark. After 30 min, the absorbance values were measured at 517 nm

and converted into percentage of antioxidant activity. Ascorbic acid (AA) was used as a positive control. The percentage of inhibition of DPPH (%) was calculated as follows:

% inhibition of DPPH = Diff x 100/Absorbance of control
where Diff = Absorbance of control – Absorbance of test sample

The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of % inhibitions plotted against the respective concentration.

2.5.2. *Ferric reducing power assay*

The reducing power of bark extract of the plant was determined according to the method as described by Oyaizu [23]. Aliquot (0.25 mL) of samples solution at different concentrations (ranging from 12.5 to 100 µg/mL) was mixed with 0.625 mL of 0.2 M phosphate buffer (pH 6.6) and 0.625 mL of 1% (w/v) solution of potassium ferricyanide. After mixing well, all the mixtures were warmed in a water bath at 50°C for 20 min. Then, 0.625 mL of 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged at 3000 rpm for 10 min. The supernatant (1.8 mL) was combined with 1.8 mL of distilled water, and 0.36 mL 0.1% (w/v) ferric chloride solution was added. The absorbance was measured at 700 nm with a spectrophotometer. Ascorbic acid was used as positive control. All the tests were run in triplicate and results were reported as mean ± SD.

2.5.3. *Phosphomolybdate radical scavenging activity*

The assay was based on the reduction of Mo(VI)-Mo(V) by the extracts and subsequent formation of a green phosphate/Mo(V) complex at acidic pH [24]. Each sample (0.1 mL) was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95ºC for 90 min. The mixture was cooled to room temperature and the absorbance of the solution was measured at 695 nm against a blank. The assays were carried out in triplicate and expressed as mean ± SD. The antioxidant activity was expressed as the absorbance of the sample.

2.5.4. *Determination of total phenolic content*

The concentrations of phenolic compounds in the samples of *P. alata* bark were measured according to the Folin-Ciocalteu method [25]. Briefly, the samples solution (0.5 mL) at different concentrations (ranging from 100 to 1100 µg/mL) was mixed with 2.58 mL of Folin-Ciocalteu's phenol reagent. After 3 min, 0.3 mL of saturated sodium carbonate solution was added to the mixture. The reaction mixtures were incubated at room temperature (25°C) for 20 min. The absorbance was measured at 760 nm with a spectrophotometer. Gallic acid solutions with concentrations ranging from 25 to 400 µg/mL were used for calibration. A dose response linear regression was generated by using the gallic acid standard absorbance and the levels in the samples were expressed as

gallic acid equivalents (mg of GAEs/g of extract). The estimation was performed in triplicate, and the results were expressed as mean ± SD.

2.5.5. *Determination of total flavonoid content*

The total flavonoid content was estimated by aluminium chloride method [26]. Plant samples (0.5 mL) were mixed with 2.5 mL of distilled water and 150 µL $NaNO_2$ solution (5%). The contents were vortexed for 10 sec and left at room temperature for 5 min. Then, 300 µL $AlCl_3$ (10 %), 1 mL NaOH (1mM) and 550 µL of distilled water were added. The solution was mixed well and kept for 15 min. The absorbance for each sample was measured at 510 nm. Quercetin concentrations ranging from 25 to 400 µg/mL were prepared and the standard calibration curve was obtained. The total flavonoid content was calculated using standard quercetin calibration curve. The results were expressed as milligrams of quercetin equivalents (QE) per gram of extract.

2.6. *Statistical analysis*

The statistical analyses were performed by a one-way ANOVA and the Student's t-test. Free R-software version 2.15.1 (http://www.r-project.org/) and Microsoft Excel 2007 (Roselle, IL, USA) were used for the statistical and graphical evaluations. The results were expressed as mean ± SD from three separate observations.

3. Results

3.1. *Phytochemical screening*

The phytochemical screening of crude ethanolic extract (CEE) of bark revealed the presence of different types of secondary metabolites, namely alkaloids, tannins, glycosides, terpenes and flavonoids (Table 1). However, CEE of bark showed the absence of saponin.

Table 1. Preliminary phytochemical study of crude ethanolic extract (CEE) of bark of *P. alata (*Roxb).

Phytochemical test	CEE
Saponins	-
Tannins	+
Glycosides	+
Steroids	+
Alkaloids	+
Flavonoids	+

CEE = crude ethanol extract, (+) = present and (-) = Not present.

3.2. *DPPH free radical scavenging activity of the plant*

For CHF and EAF fractions, good DPPH free radical scavenging activity was observed. However, the scavenging effect of CEE, HF and AQF fractions was weak (Fig. 1). IC_{50} values for CEE, HF, CHF, EAF and AQF fractions were 98.12, 105.14, 52.25, 75.01 and 172.05 respectively. For crude extract and all fractions, the scavenging activity was increased with concentration (Fig. 1).

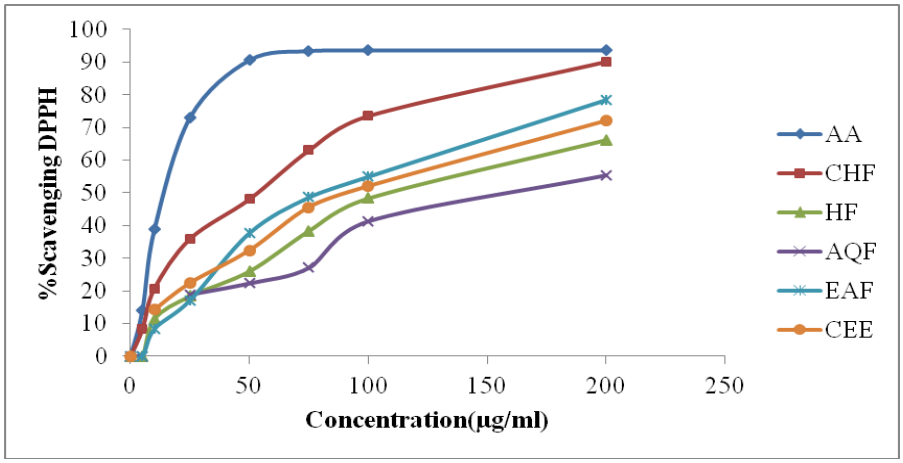

Fig. 1. DPPH radical scavenging activity of crude ethanol extract and different fractions of bark at different concentration.

3.3. *Reducing power of the plant*

Reducing activity of crude extract and different fractions were presented against concentrations in Fig. 2. The reducing power of the extract and their fractions was increased gradually with the increase in concentrations. Among the extract/fractions of leaves, the highest activity was found in CHF followed by EAF, CEE, AQF and HF (Fig. 2). At higher test concentration (100 µg/mL) the absorbance of CHF was 1.134 ± 0.021 as compared with the absorbance of AA (3.886) while rest of the fractions and CEE showed absorbance in the range of 0.963 to 0.083 (Fig. 2). Higher absorbance is an indicator of high reducing power.

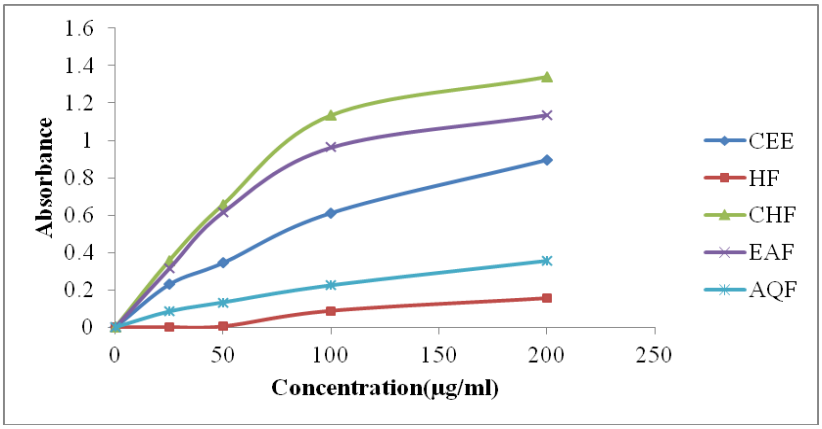

Fig. 2. Reducing power of the crude ethanol extract (CEE) and various fractions of bark

3.4. *Phosphomolybdate radical scavenging activity of the plant*

The total antioxidant capacity was measured by phosphomolybdate radical scavenging test. For crude extract and all fractions phosphomolybdate radical scavenging activity was found increased with higher concentrations. Highest phosphomolybdate radical scavenging activity was found for CHF fraction (Fig. 3). The crude extract also showed significant phosphomolybdate radical scavenging activity.

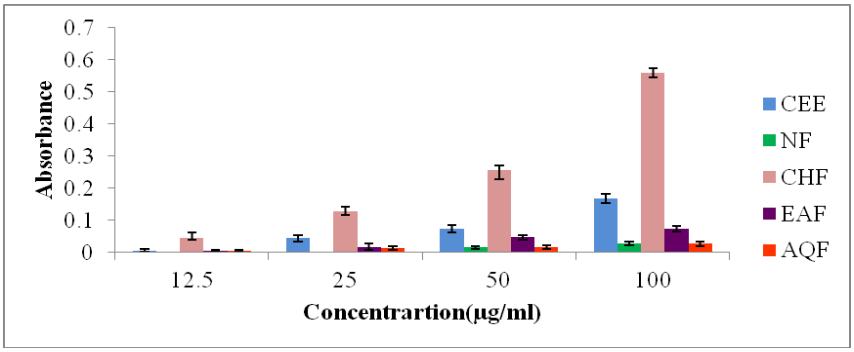

Fig. 3. Comparison of total antioxidant activity of crude ethanol extract (CEE) and various fractions of bark of the plant**.** Each value is expressed as mean ± SD (*n* = 3).

3.5. *Total phenolic and flavonoid content of crude extract and different fractions*

The total phenolic content (TPC) and total flavonoid content (TFC) of CEE and various fractions of bark were shown in Table 2. Although, crude ethanol extract and all fractions

showed the presence of TPC, the highest amount was found in CHF (29.898 µg/mL) followed by EAF (15.88 µg/mL), CEE (9.24 µg/mL), HF (9.10 µg/mL) and AQF (1.52 µg/mL) (Table 5). In case of TFC, highest content also detected in CHF (107.56 µg/mL) but followed by CEE (98.66 µg/mL), EAF (54.8 µg/mL), AQF (42.73 µg/mL) and HF (26.4 µg/mL) (Table 2).

Table 2. Total phenolic and flavonoid content of CEE and different fractions (HF, CHF, EAF and AQF) of *P. alata* (Roxb) bark.

Sample	Total Phenolic Content	Total Flavonoid Content
CEE	9.24 ± 0.772	98.66 ± 10.866
HF	9.10 ± 1.017	26.4 ± 3.966
CHF	29.898 ± 0.941	107.56 ± 5.095
EAF	15.884 ± 1.928	54.8 ± 5.651
AQF	1.52 ± 0.873	42.73 ± 5.316

CEE= Crude ethanol extract, HF= n-Hexane fraction, CHF= Chloroform fraction, EAF= Ethyl acetate fraction, AQF= Aqueous fraction. Each value is expressed as mean ± SD (n = 3).

4. Discussion

Assays based upon the use of DPPH free radical scavenging activity is the most popular spectrophotometric methods for determination of the antioxidant capacity of food, beverages and vegetable extracts [27]. CHF fraction of crude ethanol extract of bark of *P. alata* showed efficient DPPH free radical scavenging activity followed by EAF (Fig. 1). Reduction reaction antagonizes oxidation and compounds that have reduction property can protect oxidation. Both CHF and EAF showed efficient reducing power (Fig. 2). However, at total antioxidant activity determination by phosphomolybdate radical scavenging test, among all fractions, only CHF fraction showed efficient antioxidant activity. EAF fraction showed weaker antioxidant activity followed by HF and AQF.

Plant polyphenols (phenolic and flavonoid compounds) with antioxidant capacity could scavenge reactive chemical species as well as minimize oxidative damage [28]. Polyphenols exhibit 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 [29]. CHF fraction that showed efficient antioxidant activity was rich by both phenolic and flavonoid content and followed by EAF fraction (Table 5 and 6). TPC and TFC of HF and AQF were not significient. Suggesting, polyphenols of CHF are responsible for its antioxidant activity.

However, further studies are warranted to identify the exact compound(s) responsible for its antioxidant property and toxicological investigation.

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References

1. B. Halliwell, Nutr. Rev. **55**, 44 (1997). http://dx.doi.org/10.1111/j.1753 4887.1997.tb06100.x
2. K. Rajamani, T. Manivasagam, P. Anantharaman, T. Balasubramanian, and S. T. Somasundaram, J. Appl. Phycol. **23**, 257 (2011).
3. B. Uday, Curr. Sci. **77**, 658 (1999).
4. T. P. A. Devasagayam, K. K. Boloor, and T. Ramsarma, Indian J. Bioche. Biophys. **40**, 300 (2003).
5. A. H. Goldfarb, Med. Sci. Sport. Exer. **25**, 232 (1993).
6. S. Sen, R. Chakraborty, C. Sridhar, Y. S. R. Reddy, and D, Biplab, Int. J. Pharmaceut. Sci. Rev. Res. **3**, 91 (2010).
7. S. Hercberg, Am. J. Clin. Nutr. **81**, 218S (2005).
8. M. Meydani, Am. J. Clin. Nutr. **71**, 1665S (2000).
9. H. P. Wichi, Food. Chem. Toxicol. **26**, 717 (1988).
10. I. Gülçin, D. Berashvili, A. Gepdiremen, J. Ethnopharmacol. **101**, 287 (2005).
11. C. Sanchez-Moreno, J. A. Larrauri, and F. Saura-Calixto, Food Res. Int. **32**, 407 (1999).
12. B. Halliwell, and J. M. C. Gutteridge, Free Radicals in Biology and Medicine. (Oxford University Press 1999).
13. Y. Kiselova, I. Diana, C. Trifon, G. Daniela, G. Bistra, and Y. Tatyana, Phytother. Res. **20**, 961 (2006). http://dx.doi.org/10.1002/ptr.1985
14. M. Iqbal, Y. Okazaki, and S. Okada, Mol. Cell Biochem. **324**, 157 (2009). http://dx.doi.org/10.1007/s11010-008-9994-z
15. I. I. Koleva, T. A. Van Beek, J. P. H. Linssen, A. D. Groot, and L.N. Evstatieva, Phytochem. Anal. **13**, 8 (2002). http://dx.doi.org/10.1002/pca.611
16. D. Mantle, F. Eddeb, and A.T. Pickering, J. Ethnopharmacol. **72**, 47 (2000). http://dx.doi.org/10.1016/S0378-8741(00)00199-9
17. J. M. Oke and M. O. Hamburger, African J. Biomed. Res. **5**, 77 (2002).
18. R. Rastogi, B.N. Mehrotra B.N, Compendium of Indian Medicinal plants. Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi, India. **1,** (1990).
19. M. Yusuf, J. Begum, M. N. Hoque, J. U. Chowdhury, Medicinal plants of Bangladesh. BCSIR Chittagong, Bangladesh. (2009). pp. 794.
20. N. Dohou, K. Yamni, S. Tahrouch, L. M. Massani, A. Badoc, Bull. Pharm. Bordeau **142,** 61 (2003).
21. W. Brand-Williams, M. E. Cuvelier, and C. Berset, Lebensmittel-Wissenschaft und-Technol. **28,** 25 (1995).
22. E. Bursal, I. Gulcin, Food Res. Int. **44,** 1482 (2011).
23. M. Oyaizu, Jpn. J. Nutr. **44**, 307 (1986). http://dx.doi.org/10.5264/eiyogakuzashi.44.307
24. M. Umamaheswari, T. K. Chatterjee, Afr. J. Trad. Compl. Altern. Med. 2008, **5,** 61 (2008).

25. S. McDonald, P. D. Prenzler, M. Autolovich, and K. Robards, Food Chemistry. **73,** 73 (2001). http://dx.doi.org/10.1016/S0308-8146(00)00288-0
26. Kumaran, and Karunakaran, J. Food and Drug Anal. **10** (3), 178 (2007).
27. A. Bendini, L. Cerretani, L. Pizzolante, T. G. Toschi, F. Guzzo, S. Ceoldo, A. Marconi, F. Andreetta, and M. Levi, Eur. Food Res. Technol. **223**, 102 (2006).
28. I. Gulçin, D. Berashvili, and A. Gepdiremen, J. Ethnopharmacol. **101**, 287 (2005).
29. B. Halliwell, Nutr. Rev. **57,** 104 (1999).