

IN VITRO RADICAL SCAVENGING POTENTIAL OF POD AND SEED EXTRACTS OF *BAUHINIA TOMENTOSA* L.

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Received: 16 Nov 2012, Revised and Accepted: 17 Dec 2012

ABSTRACT

In the present investigation, the antioxidant and free radical scavenging efficiencies of *B. tomentosa* pod and seed extracts were evaluated for ferric reducing antioxidant power, DPPH, NO[•], hydroxyl radical scavenging, metal iron chelating, ABTS^{•+}, β-carotene-linoleate model system and antihemolytic activities. Among the samples analyzed, acetone extracts of *B. tomentosa* seed showed remarkable antioxidant activity (P<0.05) in terms of reducing power, DPPH, NO[•], metal chelating, ABTS^{•+}, β-carotene-linoleate model system and antihemolytic activities. In β-carotene-linoleic acid system, all the extracts exhibited efficient antioxidant activity and the values were comparably higher than the synthetic antioxidant, BHT and BHA. Considering all the activities performed, it is known that the seed acetone extract of *B. tomentosa* were found to be the most effective free radical quencher and a potent source of natural antioxidants due to their marked antioxidant activity. Overall, the acetone was found to be the best solvent for the extraction of antioxidant compounds. The result presented here implies that the consumption of such a legume food would not only improve the nutrient utilization but also provide the potential source of nutraceuticals for human health.

Keywords: *Bauhinia tomentosa*, Seed, DPPH, Radical scavenging activity, Lipid peroxidation.

INTRODUCTION

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, constant formation of reactive oxygen species (ROS) and oxygen-centred free radicals are witnessed in the human body by normal metabolic actions, and are one of the major implications of various pathogenesis [1]. Many acute and chronic diseases including cancer, cardiovascular troubles and neurodegenerative diseases are caused by oxidative stress initiated by these free radicals. It is believed that the maintenance of a healthy biological system depends on the balance between antioxidation and oxidation [2]. Many medicinal plants contain a broad range of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess remarkable antioxidant activity [3]. Several investigations indicate that these compounds are of great value in preventing the onset and/or progression of many human diseases [4]. The antioxidant and health-promoting capacity of these compounds are thought to arise from their protective effects by counteracting and neutralizing the ROS [3]. Therefore, searching of medicinal plants with high antioxidant content for nutritional purposes is currently of major interest.

Bauhinia tomentosa Linn. is a scrambling small tree, grows throughout southern India, Assam and Bihar. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties. The root, bark, leaves, buds, young flowers, seeds and fruits are reputed to have several medicinal properties. The dried leaves, flower buds and a decoction of the root and bark were used medicinally by the practitioners of south Africa [5,6]. Leaves are edible, sour and are used in the preparation of acid savor. In folklore medical practice, the dried leaves, buds and flower are used in dysentery and diarrhoeal affections. The bruised bark is applied externally for tumors and wounds such as scrofulous [7]. A decoction of the root bark is used in India as a vermifuge and an infusion of the stem bark as an astringent gargle. In India and Sri Lanka, the root bark is administered internally for conditions of the large intestine, and inflammation of the liver [5,8]. In spite of several medicinal properties, no information is available on the antioxidant potential of the plant. Therefore, the present study was carried out to evaluate the in vitro antioxidant potential of pod and seed extracts of *B. tomentosa*. The antioxidant activities were measured using ferric reducing antioxidant power, ABTS^{•+}, DPPH[•], NO[•], hydroxyl radical scavenging, iron chelating, β-carotene-linoleate model system and antihemolytic activity.

MATERIALS AND METHODS

Procurement and preparation of plant materials

Pod and seeds of *B. tomentosa* L. were harvested from surrounding areas of Coimbatore district, Tamil Nadu, India. The authenticity of the selected plant material was duly identified and confirmed by comparison with reference specimens preserved in the herbarium at Botanical Survey of India, Southern Circle, Coimbatore. The voucher specimens (vide no: BSI/SC/5/23/08-09/Tech.-1719) were lodged in the departmental herbarium for further reference. The plant materials were cleaned, washed with copious amounts of distilled water, shade dried, chopped into bits, and coarsely powdered in a Willy mill (Nippon Electricals, Chennai, India) to 60-mesh size for extraction.

Preparation of crude plant extracts

50 g coarsely powdered plant samples were exhaustively extracted with acetone/water (70/30, v/v), followed by methanol/water (50/50, v/v) using a round bottom flask with an attached reflux condenser for 3 h at a controlled temperature. The extracts were filtered and concentrated to dryness under reduced pressure using rotary vacuum evaporator (RE300; Yamato, Japan), lyophilized (4KBTXL-75; Vir Tis Benchtop K, New York, USA) to remove traces of water molecules and the lyophilized powders were stored at -20°C until used directly for the assessment of various *in vitro* antioxidant activities.

Reducing power

The Fe⁺⁺⁺ reducing power of the extract was determined according to the method suggested by Oyaizu [9]. Various concentrations of the extracts (dissolved in the appropriate solvents) were dissolved in 1.0 mL of phosphate buffer and 5.0 mL of 0.2 M phosphate buffer to adjust the pH 6.6. Subsequently, 5.0 mL of 1% potassium ferricyanide was added. The mixture was incubated at 50°C for 20 min and then cooled. The reaction was terminated by adding 5.0 mL of 10% TCA solution (w/v), and the mixture was centrifuged (REMI, India) at 1000 rpm for 10 min. The upper layer of the supernatant (5.0 mL) was taken and mixed with 5.0 mL of distilled water and 1.0 mL of 0.1% (w/v) ferric chloride. The absorbance was read spectrophotometrically at 700 nm against water blank. Rutin, quercetin, BHA and BHT were served as positive controls for comparison. All the tests were carried out in triplicate. A higher absorbance indicates a higher reductive capability.

DPPH radical scavenging activity

The antiradical efficiency was assessed using the DPPH[•] method as described by Blies[10]. In this method commercially available methanol soluble stable free radical DPPH was used. In its radical form, DPPH has an absorption band at 515 nm, which disappears upon reduction by an antioxidant compound or a radical species. For the photometric assay, different volumes of the extracts were taken in different test tubes. The volume was adjusted to 100 µL with methanol. 5.0 mL of 0.1 mM methanolic solution of DPPH[•] was added to these tubes and shaken vigorously. The tubes were allowed to stand for 20 min at 27°C. The control was prepared as above but without the test extract and methanol was used for the baseline correction. Changes in the absorbance of the samples were monitored at 517 nm. Results were compared with the activity of rutin, quercetin, BHA and BHT. The per cent DPPH[•] discoloration of the samples was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{517} \text{ of control} - A_{517} \text{ of sample}) / A_{517} \text{ of control}] \times 100.$$

Antioxidant activities of the extracts were expressed as IC₅₀, (the microgram of extract to scavenge 50% of the DPPH radicals) and were obtained by interpolation from linear regression analysis. A lower IC₅₀ value indicates greater antioxidant activity.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined according to the method suggested by Sreejayan and Rao[11]. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ions, which can be estimated using the Griess reagent. Scavengers of nitric-oxide act against oxygen, leading to reduced production of nitrite ions. In brief, 3.0 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with different concentrations of the extract and incubated at 25°C for 150 min. 0.5 mL of the incubated solution was removed and diluted with 0.5 mL of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylenediamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference to standards. IC₅₀, an inhibitory concentration was estimated from the % inhibition plot.

Hydroxyl radical scavenging activity

The scavenging activity for the sample extracts on hydroxyl radical was measured according to the method of Klein *et al.*[12]. 20 µg concentration of the extract was added with 1.0 mL of iron - EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) sequentially. The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and incubated at 80-90°C for 15 min in a water bath. The reaction was terminated by the addition of 1.0 mL of ice - cold TCA (17.5% w/v). Then, 3.0 mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2.0 mL of acetyl acetone were mixed and raised to 1L with distilled water) was added and left at laboratory temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured spectrophotometrically at 412 nm against reagent blank. Results were compared with the activity of standard antioxidants viz., rutin, quercetin, BHA and BHT. The % hydroxyl radical scavenging activity (HRSA) was calculated using the following formula:

$$\text{HRSA (\%)} = [A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}] \times 100.$$

Chelating ability for ferrous ions

The ferrous chelating potential of the extracts were assessed according to the method suggested by Yamaguchi *et al.*[13]. The reaction was initiated with the sequential addition of 250 µg of sample extract, 0.25 mL of 1 mM FeSO₄ solution, 1.0 mL of 0.2 M Tris-HCl buffer (pH 7.4), 1.0 mL of 2, 2' bipyridyl solution, 0.4 mL of

10% hydroxylamine hydrochloride and 2.0 mL of ethanol. The final volume was made up to 5.0 mL with deionized water and the absorbance was determined at 522 nm. EDTA was used to benchmark the chelating abilities. Lower absorbance of the reaction mixture indicated higher ferrous ion chelating ability. Results were expressed as mg EDTA equivalent/ g sample extracts.

Trolox equivalent antioxidant capacity (TEAC) assay

Antioxidant activity was performed using an improved ABTS^{•+} method proposed by Siddhuraju and Manian[14]. The ABTS radical cation (ABTS^{•+}) was generated by a reaction of 7 mmol/ L ABTS and 2.45 mmol/ L potassium persulfate after incubation for 16 h at laboratory temperature in dark. Blue - green ABTS^{•+} was formed at the end of this period. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to obtain an absorbance of 0.700 ± 0.02 at 734 nm, the wavelength of maximum absorbance in the visible region. The stock solution of the sample extracts in ethanol was diluted such that, after introduction of a 10 µL aliquot of each dilution into the assay, they produced between 20- 80% inhibition of the blank absorbance. After the addition of 1.0 mL of diluted ABTS^{•+} solution to 10µL of sample extracts or Trolox standards (final concentration 0-15 µM) in ethanol, absorbance was recorded at 30°C, exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicates were maintained for the experiments and the per cent inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration[15]. The unit of total antioxidant activity (TAA) was defined as the concentration of Trolox having the equivalent antioxidant activity expressed as µmol/ g sample extracts on dry weight basis.

Inhibition of β - carotene bleaching

The antioxidant capacity of the extract was evaluated using β - carotene-linoleate model system[16]. 1.0 mg of β - carotene was dissolved in 10 mL of chloroform and mixed with 20 mg of linoleic acid and 200 mg of Tween - 40 emulsifier mixture. Chloroform was completely removed at 45°C under vacuum using a rotary vacuum evaporator. 50 mL of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion. A 5.0 mL aliquot of the emulsion was dispensed into tubes containing 100 µg/ mL of the sample extract. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. Subsequent absorbance readings were recorded at 15 min intervals by keeping the sample tubes in a water bath at 50°C until the visual colour of β - carotene in the control sample disappeared (about 120 min). A blank, devoid of β - carotene was prepared for background subtraction. Rutin, quercetin, BHA and BHT were used as standards. All determinations were performed in triplicate and averaged.

The antioxidant activity (AA) was measured in terms of reduction in β - carotene bleaching activity using the following formula:

$$\text{AA (\%)} = [1 - (A_s^0 - A_s [12]^0) / (A_c^0 - A_c [12]^0)] \times 100$$

Where, A_s⁰ is the absorbance of sample at 0 min, A_s [12]⁰ is the absorbance of sample at 120 min, A_c⁰ is the absorbance of control sample at 0 min and A_c [12]⁰ is the absorbance of control sample at 120 min.

Antihemolytic activity

The preparation of erythrocyte membrane ghost and the subsequent determination of the antioxidant activity of the extracts on the chemically induced lipid peroxidation were performed according to the method set forth by Naim *et al.*[17]. The erythrocytes from cow blood were separated by centrifugation (2000 rpm for 10 min) and washed with saline phosphate buffer (0.9 g of sodium chloride dissolved in 100 mL of 0.2 M phosphate buffer of pH 7.4) until the supernatant becomes colourless. The erythrocytes were then diluted with saline phosphate buffer to give 4% (v/v) suspension. 500 µg of extract/ mL of saline phosphate buffer was added to 2.0 mL of erythrocyte suspension and the volume was made up to 5.0 mL with saline phosphate buffer. This mixture was pre-incubated for 5 min and then 0.5 mL of H₂O₂ solution of appropriate concentration in

saline buffer was added. The concentration of H₂O₂ in the reaction mixture was adjusted so as to bring about 90% hemolysis of blood cells after 240 min. After the incubation time, the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of hemolysis was determined by measurement of the absorbance (at 540 nm) corresponding to haemoglobin liberation. Natural and synthetic standards at the same concentration as sample extract were used for comparison.

The percent hemolysis inhibition was calculated using the formula:

$$\text{Inhibition percentage} = \left[\frac{A_{\text{control}} - A_{\text{Sample}}}{A_{\text{control}}} \right] \times 100.$$

Statistical analysis

For *in vitro* antioxidant activity of the extracts, the results were recorded as mean \pm standard deviation (SD) (n = 3) and subjected to one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range test using SPSS (version 9, SPSS Inc., Chicago, USA). P < 0.05 was chosen as the criterion for statistical significance.

RESULTS AND DISCUSSION

Reductive ability of *B. tomentosa* pod and seed extracts

In this assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of test specimen. The presence of reductants (antioxidants) might cause the reduction of Fe³⁺/ Ferric cyanide complex to ferrous form. The ferrous ion can therefore be monitored by measuring the formation of Per's Prussian blue at 700 nm[18]. Fig. 1 shows the dose- response curves of *B. tomentosa* pod and seed extracts. Their reductive abilities displayed an apparent linear relationship with concentration. At the same concentration (20-100 μ g/ mL) tested, seed acetone extracts displayed remarkably higher reducing abilities which surpassed the efficiency of all the natural and synthetic antioxidants used in the study. Similarly the pod acetone extracts also manifested stronger activity. Accordingly, it can be suggested that the polyphenolic richness of the extracts might appear to function as good electron and hydrogen atom donors and therefore could terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products[19]. Likewise, Oktay *et al.*[20] suggested that there may be highly significant positive relationship between total phenols and antioxidant activity in many plant species.

DPPH-scavenging abilities of *B. tomentosa* pod and seed extracts

DPPH is a stable nitrogen-centered free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Substances which perform the above reaction can be considered as antioxidants and therefore radical scavengers[21]. The concentration of an antioxidant needed to decrease the DPPH concentration by 50% (IC₅₀) is a parameter widely used to estimate antioxidant activity[22]. In the present study, acetone seed extracts of *B. tomentosa* presented significantly (P < 0.05) higher activity than those of other solvent extracts used (Table 1). However higher levels of DPPH activity observed in the crude acetone extracts of *B. tomentosa* seed might be due to the presence of biomolecules with pronounced antioxidant activity. Interestingly, their scavenging ability was relatively higher than the positive controls tested. Amarowicz *et al.*[23] also reported that the tannins extracted from canola and rapeseed hulls exhibited a high scavenging efficiency toward DPPH radicals.

NO[•] scavenging abilities of *B. tomentosa* pod and seed extracts

Virtually all cellular components including lipids, proteins, nucleic acids, carbohydrates are susceptible to oxidative damage[24]. ROS like O₂^{•-} may react with NO and give rise to various other reactive nitrogen species (RNS) such as NO₂, N₂O₄ and peroxy nitrite. Both ROS and RNS together attack and damage various cellular molecules, resulting in several pathological conditions including cancer[25]. A number of polyphenolic phytochemicals such as resveratrol, quercetin, α -tocopherol and catechin have been found to inhibit the RNS effect[26]. Therefore, utilization of these significant sources

of natural antioxidants to prevent or improve ROS or RNS mediated injury becomes very important. In the present study, nitrite ions generated by sodium nitro prusside (SNP), in aqueous solution at physiological pH was extensively scavenged by *B. tomentosa* extracts in a concentration dependent manner (Table 1). Among the samples analyzed, the crude seed acetone extracts favorably quenched NO and it compared well with all the standard antioxidants tested. This preferential NO scavenging potential of the extract could be attributed to its higher flavonoid contents. van Acker *et al.*[27] made similar observation that flavonoids play a major role in the stabilization of NO. Since the flavonoids are known to have antioxidant properties, the presence of high flavonoid contents in these species could be the basis for the observed radical scavenging activity.

Metal ion chelating activities of *B. tomentosa* pod and seed extracts

Transition metal ions, particularly iron is an essential mineral for normal physiology, but excess can result in cellular injury. If they undergo the Fenton reaction, the reduced metals may form highly reactive hydroxyl radicals and thereby contribute to oxidative stress[28]. The resulting oxyradicals cause damage to cellular lipids, nucleic acids, proteins, and carbohydrates and lead to cellular impairment. Since ferrous ions are the most effective pro-oxidants in food systems, their removal from circulation could be one of the promising approach to prevent oxidative stress-induced diseases. Several plant extracts/ constituents have been reported to exert antioxidant activity by chelating the catalytic metals[29]. In the present investigation, it was observed that all the assessed sample extracts of *B. tomentosa* offered pronounced antioxidant activities as they were able to chelate ferrous metal ion more efficiently (Table 1). Apparently the acetone extracts of *B. tomentosa* seed exhibited excellent chelating ability for ferrous ions and might afford protection against oxidative damage. The iron chelating properties of the extract might be attributed to their endogenous chelating agents, especially phenolics. It is speculated that the properly oriented functional groups of certain phenolics, tannins and flavonoids might act effectively as the antioxidant agents[30]. Accordingly it is suggested that the ferrous ion chelating effects of these fractions would be beneficial in protecting against the adverse effects of oxidative damage.

Total antioxidant activities of *B. tomentosa* pod and seed extracts

In TEAC (Trolox equivalent antioxidant capacity) method, all the assessed samples of *B. tomentosa* were able to quench ABTS^{•+} radical more efficiently with their TEAC values ranging between 658.1 to 5744.2 μ mol Trolox equivalent/ g extract (Table 1). Among the samples investigated, the seed acetone extracts of *B. tomentosa* were found to be the excellent source to scavenge ABTS radicals generated in the system and the antioxidant activity of such samples seems to be sufficient for functioning as potential nutraceuticals when they are ingested along with nutrients. The highest antioxidant power of the acetone extracts may be contributed by the hydrogen-donating compounds likely to be present in the polar solvents[31]. Different ratios of water in the polar solvent systems (70% acetone and 50% methanol) also differed in the ABTS radical scavenging activities of the samples, indicating that the extracting solvent composition also significantly influenced the antioxidant activity, with a varying mode of functions. The polyphenolic-rich extracts extracted from polar solvent, especially 70% acetone might act as a potent donors of hydrogen by forming resonance - stabilized phenoxyl radicals[32]. Hagerman *et al.*[33] have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS^{•+}) and that effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group substitution, rather than their specific functional groups.

Hydroxyl radical scavenging activity of *B. tomentosa* pod and seed extracts

Hydroxyl radical is the most dangerous radical in the body. It can be formed from superoxide anion and hydrogen peroxide in the

presence of metal ions such as copper or iron. Hydroxyl radicals have the highest 1-electron reduction potential (2310 mV) and can react with everything in living organisms at the second-order rate constants of 10^9 – 10^{10} mol/s. It reacts with lipids, polypeptides, proteins, and DNA molecules, especially thiamine and guanosine. When a hydroxyl radical reacts with aromatic compounds, it can add across a double bond, resulting in hydroxycyclohexadienyl radical. The resulting radical can undergo further reactions, such as reaction with oxygen, to give peroxy radical, or decompose to phenoxy-type radicals by water elimination[34]. A highly significant difference ($P < 0.05$) in the scavenging ability of *B. tomentosa* extracts against hydroxyl radicals generated in a Fenton reaction system was observed in the present study. It was apparent that 70% acetone extracts of *B. tomentosa* pod modestly scavenged off these free radicals and/or inhibited the generation of free radicals (Table 1). It has already been discussed that the potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution[35].

Antioxidant activity of *B. tomentosa* pod and seed extracts in the β -carotene linoleate model system

The antioxidant activity of the extracts was determined using β -carotene-linoleic acid coupled oxidation model system. β -carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β -carotene and linoleic acid, which generate free radicals. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β -carotene molecules. As a result, β -carotene will be oxidized and broken down in part; subsequently, the system loses its chromophore and the characteristic orange colour, which can be monitored spectrophotometrically. The presence of different antioxidants can hinder the extent of β -carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system[36]. Thus, the degradation rate of β -carotene – linoleate depends on the antioxidant activity of the extracts. The extract with the lowest β -carotene degradation rate exhibited the highest antioxidant activity. As shown in Fig. 2, addition of *B. tomentosa* extracts at various concentrations prevented the bleaching of β -carotene to various degrees by neutralizing the linoleate free radical and other free radicals formed in the system. In comparison, the seed acetone extracts exhibited an appreciable

antioxidant activity at 100 $\mu\text{g}/\text{mL}$, which often surpassed the performance of the natural and synthetic antioxidants tested (Fig. 2). It is believed that lipid peroxidation is one of the causes of the occurrence of cardiovascular diseases[37] and cancer[38]. The high inhibition rate of the extracts obtained in the study might therefore contribute in a big way to their therapeutic potential. Inhibition of the oxidation rate of β -carotene molecules in the present study might be due to the presence of the relative concentration of phenolic compounds in the extracts. Siddhuraju and Becker[39] have also reported that the presence of a relatively higher concentration of polyphenolic compounds, particularly tannins and flavonoids, in the acetone and methanol extracts was proved to have more antioxidant power.

Anti-hemolytic activity of *B. tomentosa* pod and seed extracts

Plant extracts and plant-derived antioxidants are receiving wide attention in food industry and in biomedical research primarily as a result of their ability to stabilize bulk oils, emulsions, and biological membrane against lipid peroxidation[40]. Hemolysis has a long history of use in measuring free radical damage and its inhibition by antioxidants, but only few studies have been performed with erythrocytes in whole blood[41]. Moreover, the RBC hemolysis is a more sensitive system for evaluating the antioxidant properties of the phytochemicals. As shown in Fig. 3, the protective effect of *B. tomentosa* on hemolysis of cow blood erythrocytes scattered widely between plant parts and solvent types. This expected variation might be due to the complexity and extremely high polarity of their antioxidant chemical compositions. Furthermore, the acetone extracts of *B. tomentosa* seed offered more protection against erythrocyte hemolysis when compared with the other studied components. It is speculated that the pronounced antioxidant activity of the highly polar extracts must stem from the action of native bioantioxidants, such as phenolics, flavonoids and tannins present in these extracts, which in turn might inactivate lipid free radicals or prevent decomposition of hydroperoxides into free radicals. Similarly, Chestnut skins also showed a high protective effect against erythrocyte hemolysis (outer skin: 95.7%, inner skin: 92.7%; at 1 mg/ mL) as reported by Barreira *et al.*[42]. The present study thus proves that the test plant extracts, apart from having an excellent radical quenching property, also possess protective capability against hemolysis, possibly due to high concentration of polyphenolic constituents.

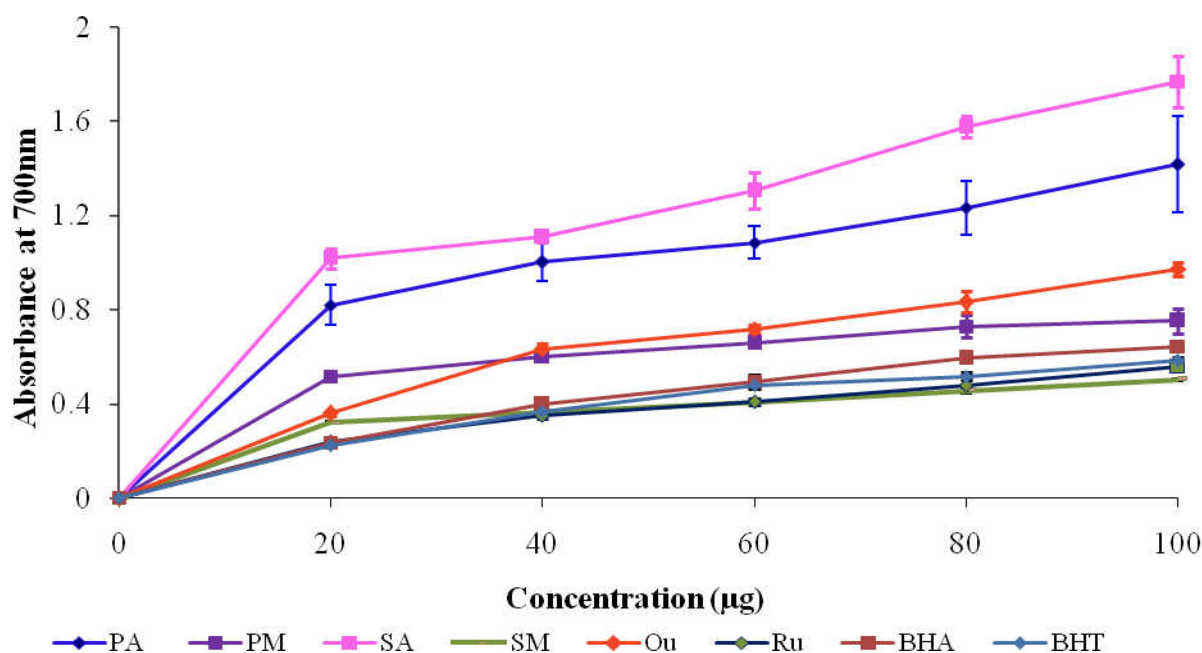


Fig. 1: Reductive capability of different parts of *B. tomentosa* compared with standard antioxidants.

PA – Pod Acetone, PM- Pod Methanol, SA- Seed Acetone, SM – Seed Methanol, Ou – Quercetin, Ru – Rutin.

Values are presented as the mean \pm standard deviation of three independent experiments.

Table 1: DPPH scavenging, nitric oxide scavenging, hydroxyl scavenging, total antioxidant activity (TAA), and ferrous ion chelating ability of pod and seed extracts of *B. tomentosa*.

Plant parts	IC ₅₀ values (µg/ml)		Hydroxyl Scavenging activity (%)	Metal chelating activity [#]	ABTS*
	DPPH*	NO*			
PA	45.9 ± 1.7 ^g	53.2 ± 2.3 ^e	37.9 ± 5.8 ^b	100.6 ± 0.3 ^b	1879.9 ± 400.5 ^b
PM	115.3 ± 9.5 ^h	71.3 ± 0.6 ^f	16.2 ± 0.9 ^e	72.7 ± 1.6 ^d	1451.2 ± 168.6 ^c
SA	18.4 ± 0.4 ^b	30.4 ± 3.7 ^a	12.9 ± 0.8 ^f	280.2 ± 1.9 ^a	5744.2 ± 660.6 ^a
SM	32.5 ± 2.8 ^e	43.9 ± 2.4 ^c	25.9 ± 2.3 ^d	84.1 ± 0.6 ^c	658.1 ± 484.4 ^d
Ru	15.8 ± 0.01 ^a	42.1 ± 0.03 ^c	15.7 ± 0.8 ^e	-	-
Qu	20.7 ± 0.1 ^c	50.8 ± 4 ^d	34.9 ± 3.5 ^c	-	-
BHA	21.4 ± 0.1 ^d	52.9 ± 8 ^e	35.5 ± 1.9 ^c	-	-
BHT	34.7 ± 0.3 ^f	38.5 ± 1 ^b	45.6 ± 0.8 ^a	-	-

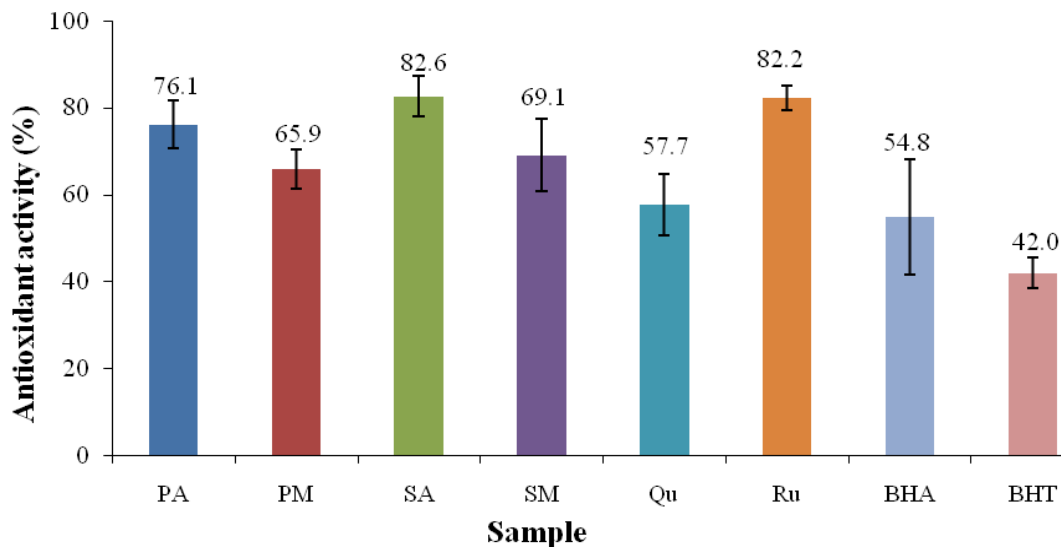
PA – Pod Acetone, PM – Pod Methanol, SA – Seed Acetone, SM- Seed Methanol, Ru – Rutin, Qu – Quercetin.

[#]Values expressed in mg EDTA / g extract.

* Values expressed as TEAC (Trolox equivalent antioxidant capacity) in µ mol/g extract.

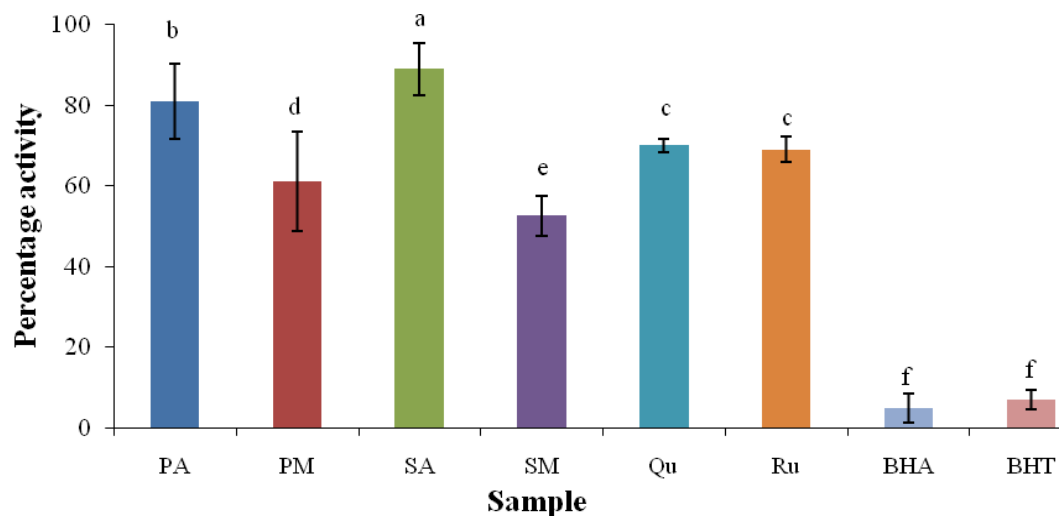
Values are presented as the mean ± standard deviation of three independent experiments.

Mean values not sharing a common letter in a column were significantly different (P < 0.05).

**Fig. 2: Lipid peroxidation preventive property of pod and seed extracts of *B. tomentosa* in β-carotene-linoleic acid system.**

PA – Pod Acetone, PM – Pod Methanol, SA – Seed Acetone, SM- Seed Methanol, Qu – Quercetin, Ru – Rutin.

Values were presented as the mean ± standard deviation of three independent experiments. Vertical bars labeled with different letters are significantly different (P < 0.05).

**Fig. 3: Antihemolytic property of pod and seed extracts of *B. tomentosa*.**

PA – Pod Acetone, PM – Pod Methanol, SA – Seed Acetone, SM- Seed Methanol, Qu – Quercetin, Ru – Rutin.

Values were presented as the mean ± standard deviation of three independent experiments. Vertical bars labeled with different letters are significantly different (P < 0.05).

CONCLUSION

To conclude, *B. tomentosa* seed extracts presented a broad range of antioxidant activities but with different efficacies. Among the samples analyzed, acetone extract of *B. tomentosa* seed determined to have highest range of antioxidant activity in terms of the assays used for the determination of antioxidant potential. Presence of high contents of polyphenolics might be the major contributor of antioxidant capacities of these plant samples. However, further investigations are needed to establish the exact mechanism of action and to identify the active bio-ingredient(s) of the extract in order to explain their therapeutic efficacy.

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