FREE RADICAL SCAVENGING ABILITIES OF LEAF, STEM BARK AND ROOT EXTRACTS OF BAUHINIA TOMENTOSA L. (CAESALPINIACEAE)

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ABSTRACT
Crude extracts of leaf, stem bark and root parts of edible and therapeutically important plant, Bauhinia tomentosa L. were analyzed for their antioxidant and free radical scavenging properties. The extracts of different fractions were found to have different levels of antioxidant activity in the in vitro systems tested. In the present investigation, the aqueous acetone extracts of leaf showed remarkable antioxidant activity in terms of ferric reducing power, metal chelation, β-carotene-linoleic acid model system and antihemolytic activities. Considering all the activities performed, it is known that the leaf acetone extract of B. tomentosa L. was found to be the most effective free radical quencher and a potent source of natural antioxidants, thus justifying their traditional claim.

KEYWORDS: Bauhinia tomentosa, leaf, ABTS**, DPPH*, radical scavenging activity, lipid peroxidation.

INTRODUCTION
In last few decades, there has been an increasing interest in medicinal plants for their phenolic concentration and related total antioxidant potential (Katalinic et al., 2006). It has been reported that many medicinal plants contain a broad range of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess potent antioxidant activity (Wong et
Several investigations indicate that these compounds are of great value in preventing the onset and/or progression of many dreadful diseases (Halliwell et al., 1992).

The antioxidant and health-promoting capacity of the plants are thought to arise from their protective effects by counteracting and neutralizing the reactive oxygen species (ROS) (Wong et al., 2006). The genus, Bauhinia belongs to the family, Caesalpiniaaceae has been studied extensively in recent years for its medicinal values (The Wealth of India, 1988). Phytochemical investigations of the genus have revealed the presence of a number of compounds including steroids, glycosides, triterpenes, lactones and flavonoids (Manivannan et al., 2010). Biological studies have confirmed that these plants exert several medicinal properties, especially antinociceptive, antimicrobial and antidiabetic effects (Meyre-Silva, 2004).

*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 were reported to possess several medicinal properties. In Ayurveda, its parts are recommended in a combined form with other drugs for the treatment of snake bite and scorpion-sting (The Wealth of India, 1988). The dried leaves, flower buds and a decoction of the root and bark were used medicinally by the doctors of South Africa (Bhattacharjee, 2004). Leaves are edible, sour and are used for the preparation of acid sayor. In folklore medical practice, the dried leaves, buds and flowers are used in dysentery and diarrhoeal affections. The bruised bark is applied externally for tumors and wounds such as scrofulous (Manivannan et al., 2010). In India, 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 (Singh and Panda, 2005). In spite several medicinal properties, no information is made available on the antioxidant potential of the plant. Therefore, the present study was carried out to evaluate the *in vitro* antioxidant potential of leaf, stem bark and root extracts of *B. tomentosa* L. The antioxidant activities were measured using ferric reducing power, ABTS+, DPPH*, NO’, hydroxyl radical scavenging, iron chelating, β– carotene- linoleate model system and antihemolytic activities.
MATERIALS AND METHODS

Procurement and preparation of plant materials
Fresh leaves stem and root parts of *Bauhinia tomentosa* L. were harvested from the 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 specimen (vide no: BSI/SC/5/23/08-09/Tech.-1719) was 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 of coarsely powdered plant samples were exhaustively extracted with petroleum ether, ethyl acetate, acetone/water (70/30, v/v), followed by methanol/water (50/50, v/v) using soxhlet apparatus 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 last traces of water molecules and stored at -20°C until used directly for the assessment of various in vitro antioxidant activities.

In vitro antioxidant potential of *B. tomentosa* L. extracts
Reducing power
The Fe^{+++} reducing power of the extract was determined according to the method suggested by Oyaizu (1986). Various concentrations of the extracts (dissolved in 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 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 DPPH’ method as described by Blois (1958). 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 27ºC. 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 % DPPH’ discoloration of the samples was calculated using the following formula:

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

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

Nitric oxide scavenging activity
Nitric oxide scavenging activity was determined according to the method suggested by Sreejayan and Rao (1997). 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. IC\textsubscript{50}, an inhibitory concentration was estimated from the % inhibition plot.
Chelating ability for ferrous ions

The ferrous chelating potential of the extracts were assessed according to the method suggested by Yamaguchi et al. (2000). The reaction was initiated with the sequential addition of 250 µg of sample extract, 0.25mL of 1Mm 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 (2007). 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 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 10 µL 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 experiment and the per cent inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration (Re et al., 1999). 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 a dry weight basis.

Hydroxyl radical scavenging activity

The scavenging activity for the sample extracts on hydroxyl radical was measured according to the method of Klein et al. (1991). 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:

\[
\text{HRSA} (%) = \left[ \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100.
\]

**Inhibition of β-carotene bleaching**

The antioxidant capacity of the extract was evaluated using β-carotene-linoleate model system (Taga *et al.*, 1984). 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. 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:

\[
\text{AA} (%) = \left[1 - \frac{(A_s^0 - A_s^{120})}{(A_c^0 - A_c^{120})} \right] \times 100
\]

Where, \(A_s^0\) is the absorbance of sample at 0 min, \(A_s^{120}\) is the absorbance of sample at 120 min, \(A_c^0\) is the absorbance of control sample at 0 min, and \(A_c^{120}\) 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. (1976). 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 were 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 H$_2$O$_2$ solution of appropriate concentration in saline buffer was added. The concentration of H$_2$O$_2$ 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 extend 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 per cent 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

The results were recorded as mean ± 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

Fig. 1: Reductive capability of standard antioxidants.

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

Fig. 2: Reductive capability of various solvent extracts of *B. tomentosa* L. leaf.

*Values were presented as the mean ± standard deviation of three independent experiments. Refer Table 1 for abbreviation expansions.
Fig. 3: Reductive capability of various solvent extracts of *B. tomentosa* L. stem.

Values were presented as the mean ± standard deviation of three independent experiments. Refer Table 1 for abbreviation expansions.

Fig. 4: Reductive capability of various solvent extracts of *B. tomentosa* L. root.

Values were presented as the mean ± standard deviation of three independent experiments. Refer Table 1 for abbreviation expansions.
The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free – radical chain through donation of hydrogen atom. Some studies have reported that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Jeong et al., 2004). The Fe$^{3+}$ - Fe$^{2+}$ transformation in the presence of different solvent extracts of B.tomentosa L. were investigated. Fig 1-4 shows the dose- response curves for the reducing power of various solvent extracts obtained from different parts of B.tomentosa L. Their reductive abilities displayed an apparent linear relationship with concentration. The activity increases exponentially with increasing concentration of the test drug.

Among the solvent systems examined, acetone extracts provided higher reductive power values. It must be emphasized that the acetone extracts of leaf exerted stronger reducing abilities than their corresponding extracts. Similarly the acetone extracts of root and stem also manifested stronger activity. From the present report it can be established that the polyphenolic richness of the extracts might appear to function as good electron and hydrogen atom donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products. Similar observation between the polyphenolic constituents in terms of dose dependent and reducing power activity have been reported for several plant extracts including tea (Amarowicz et al., 2004). Oktay et al. (2003), suggested that there may be highly significant positive relationship between total phenols and antioxidant activity in many plant species.

Kang and Saltveit (2002) suggest that DPPH radical scavenging activity is a measure of non-enzymatic antioxidant activity. Higher levels of DPPH activity have been correlated with tolerance to different stress conditions. The presence of biologically active biomolecules in the plant exerts pronounced antioxidant activity (Malencic et al., 2007). DPPH radical was used to evaluate the free radical scavenging ability of the investigated samples and their values are significantly (P<0.05) different (Table.1).A lower IC$_{50}$ value corresponds with a highest antioxidant power. In the present study, all the assessed samples were able to interact intensively with DPPH and reduce the stable violet DPPH radical to the yellow DPPH-H, reaching their 50% reductive plateau ranging between 35 ± 0.4 to 347.7±14.5μg /ml. As reference, rutin taken as positive control recorded the highest scavenging efficiency towards DPPH radicals (15.8 ±.01μg /ml), followed by quercetin (20.7±0.05 μg /ml) and BHA (21.4±0.1 μg /ml), respectively. The DPPH radical scavenging effects of the extracts were in
the order of: LA > RA > SA > LM > SM > RM. It was observed that the aqueous acetone extract was able to quench DPPH radicals more effectively than its corresponding solvent types. This trend was similar to that observed for total phenolics, tannins and total flavonoid contents in our laboratory earlier (Thenmozhi et al., 2012). This may be contributed to the fact that the hydrogen-donating compounds are more likely to be present in the polar solvents (Middleton et al., 2005). Sidduraju et al. (2002) reported that high concentration of tannins (proanthocyanidins) extracted from stem bark of Cassia fistula possessed elevated DPPH radical quenching capacity. Therefore it may be stated that B. tomentosa is a good source of free radical quencher.

NO is a free radical with a single unpaired electron. The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Furthermore, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that is detrimental to human health. In the present study, antioxidant abilities of B. tomentosa L. to scavenge nitric oxide generated in vitro by sodium nitroprusside were investigated (Table 1). All the extracts inhibited nitric oxide generation in a concentration dependent manner. However their comparative data on nitric oxide scavenging activity fluctuated between plant parts and the solvent types. It exhibited excellent to fairly outstanding antioxidant activity with their IC$_{50}$ values less than 100 μg /ml (20.7±1.5 to73.5±0.2 μg/ml). However, it was observed that the acetone and methanolic extract of B. tomentosa. stem, root, and leaf recorded markedly higher ability to scavenge NO and/or inhibits the production of nitric oxide radicals more actively as it compared well with the standard natural and synthetic antioxidants used, thus emphasizing the positive effects of polyphenolics present in it. A number of polyphenolic phytochemicals such as resveratrol and quercetin (Kawada et al., 1998), α-tocophenol (Arroyo et al., 1992) and catechin (Pannala et al., 1998) have been found to inhibit the RNS effect. Therefore utilization of these significant sources of natural antioxidants to prevent ROS or RNS mediated injury becomes very important.
Table 1: DPPH scavenging, nitric oxide scavenging, hydroxyl scavenging, total antioxidant activity (TAA) and ferrous ion chelating ability of different parts of *B. tomentosa* L.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Abbreviation</th>
<th>IC$_{50}$ (µg/mL)</th>
<th>Hydroxyl scavenging activity (%)</th>
<th>Metal chelating activity (mg EDTA/g sample extract)*</th>
<th>ABTS (µ molar Trolox equivalent/g sample extract)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DPPH</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf petroleum ether</td>
<td>LP</td>
<td>347.7 ± 14.5a</td>
<td>46.8 ± 0.01a</td>
<td>18.8 ± 1.4i</td>
<td>66.8 ± 1.1j</td>
</tr>
<tr>
<td>Leaf ethyl acetate</td>
<td>LE</td>
<td>159.8 ± 18.2k</td>
<td>34.6 ± 5.6i</td>
<td>18.4 ± 1.2i</td>
<td>52.6 ± 0.6b</td>
</tr>
<tr>
<td>Leaf acetone</td>
<td>LA</td>
<td>35 ± 0.4d</td>
<td>24.5 ± 6c</td>
<td>26.4 ± 1.5e</td>
<td>195.5 ± 1.7a</td>
</tr>
<tr>
<td>Leaf methanol extract</td>
<td>LM</td>
<td>54.8 ± 1.7g</td>
<td>26.5 ± 1.9g</td>
<td>22.1 ± 2.0g</td>
<td>89.2 ± 1.5l</td>
</tr>
<tr>
<td>Stem petroleum ether extract</td>
<td>SP</td>
<td>97.2 ± 61.5j</td>
<td>54.6 ± 3.4h</td>
<td>14 ± 1.6e</td>
<td>100.5 ± 0.9d</td>
</tr>
<tr>
<td>Stem ethyl acetate extract</td>
<td>SE</td>
<td>219.6 ± 1.1m</td>
<td>40.2 ± 8.3i</td>
<td>13.7 ± 1.4m</td>
<td>79.2 ± 1.5b</td>
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<td>Stem acetone extract</td>
<td>SA</td>
<td>52.3 ± 4.1l</td>
<td>20.7 ± 1.5a</td>
<td>30.7 ± 0.4a</td>
<td>84.4 ± 1.5a</td>
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<tr>
<td>Stem methanol extract</td>
<td>SM</td>
<td>57.3 ± 4.7h</td>
<td>26.2 ± 0.3a</td>
<td>17 ± 0.3j</td>
<td>71.9 ± 1.9l</td>
</tr>
<tr>
<td>Root petroleum ether extract</td>
<td>RP</td>
<td>333.7 ± 4.4n</td>
<td>73.5 ± 0.2a</td>
<td>14.3 ± 3.1l</td>
<td>34.6 ± 1.7j</td>
</tr>
<tr>
<td>Root ethyl acetate extract</td>
<td>RE</td>
<td>203.1 ± 3.7i</td>
<td>32.9 ± 0.8a</td>
<td>16.8 ± 1.4l</td>
<td>96.9 ± 2.2l</td>
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<tr>
<td>Root acetone extract</td>
<td>RA</td>
<td>46.5 ± 1.9c</td>
<td>22.9 ± 0.2b</td>
<td>24 ± 1.2l</td>
<td>116.5 ± 0.8b</td>
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<tr>
<td>Root methanol extract</td>
<td>RM</td>
<td>91.8 ± 0.2n</td>
<td>35.1 ± 2.9a</td>
<td>20.9 ± 1.7n</td>
<td>114.8 ± 1.5c</td>
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<td>Rutin</td>
<td></td>
<td>15.8 ± 0.01a</td>
<td>42.1 ± 0.03j</td>
<td>15.7 ± 0.8k</td>
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<td>Quercetin</td>
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<td>20.7 ± 0.05b</td>
<td>50.8 ± 4.4f</td>
<td>34.9 ± 3.5c</td>
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<tr>
<td>BHA</td>
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<td>21.4 ± 0.1c</td>
<td>52.7 ± 8m</td>
<td>35.5 ± 1.9n</td>
<td></td>
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<tr>
<td>BHT</td>
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<td>34.7 ± 0.3d</td>
<td>38.5 ± 1a</td>
<td>45.6 ± 0.9a</td>
<td></td>
</tr>
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</table>

*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).

*Values expressed as mg EDTA/g extract; *Values expressed as TEAC (Trolox equivalent antioxidant capacity) in µmol/g extract.
Hydroxyl radical is one of the most important potent oxidant among the reactive oxygen species and is considered to be an initiator of lipid peroxidation. It induces severe damage to the adjacent molecules and can be generated in biological cells through the Fenton reaction. At a concentration of 20 μg/ml, different parts of *B. tomentosa* L. exhibited fairly moderate radical scavenging activities towards the hydroxyl radicals generated (13.7 and 30.7%) in the reaction mixture (Table 1). Almost all the extracts scavenged the hydroxyl radicals in a similar extent. In similar lines, both the polar solvent extracts revealed the consistent hydroxyl radical scavenging activity. However, the acetone extracts of stem bark (30.7%) showed the highest efficiency of scavenging hydroxyl radicals and these results suggest that acetone extracts may be considered potent free radical scavengers. Among the different standard antioxidants tested, BHT (45.6%) was found to be more effective in scavenging hydroxyl radicals. The ability of the above mentioned extracts to quench hydroxyl radicals seems to be related to the prevention of lipid peroxidation and hence to be good scavenger of active free radicals, thus reducing the rate of chain reaction. It was already reported that naturally occurring phenolic compounds have free radical scavenging properties, due to their hydroxyl groups (Diplock, 1997). Furthermore, phenolic compounds are effective hydrogen donors, which make them imminent antioxidants (Rice-Evans et al., 1995).

Likewise, the chelation of ferrous ion by different parts of *B.tomentosa* L. was estimated by 2,2’-bipyridyl competition assay and their values were expressed as EDTA equivalents on a dry weight basis (Table 1). Iron is an essential mineral for normal physiology, but excess can result in cellular injury. If they undergo the Fenton reaction, these reduced metals may form highly reactive hydroxyl radicals and thereby contribute oxidative stress (Hippeli and Elstner, 1999). Ferrous ions are the most effective pro-oxidants in food systems, therefore, commendable chelating effect is considered beneficial and removal of free iron ion from circulation could be one of the promising approaches to prevent oxidative stress-induced diseases, hence, metal chelation is one of the important properties of an antioxidant. It was observed that all the solvent extracts of *B.tomentosa* L. displayed an apparent (P<0.05) antioxidant activities as they were able to chelate ferrous metal ion more efficiently with their values ranging between 34.6 and 195.5 mg EDTA/g of sample, respectively. Among them, the acetone extract of leaf was found to be the most active (195.5 mg EDTA/g sample) ferrous ion chelator. The ability of phenolic compounds to chelate metal ions depends on the availability of properly oriented functional groups (Van Acker et al., 1996). We speculate that these active principles might be responsible for the observed activity.
Hagerman et al. (1998) have recently 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 groups substitution, rather than the specific functional groups. On the other hand, the formation of tannin-protein complex, both in insoluble and soluble complexes, have also been shown to be potential free radical scavenger and radical sinks. Moreover, such complexes could also have been suggested to be one of the nutraceutical contributors to prevent the free radical mediated diseases occurring in the gastrointestinal tract (Riedl and Hagerman, 2001). The Total Equivalent Antioxidant Capacity (TEAC) was measured using the improved ABTS\(^{•+}\) radical decolorization assay. All the sample extracts of *B.tomentosa* L. species were able to quench ABTS\(^{•+}\) radical more efficiently with their TEAC values ranging between 47.2 ± 5.8 to 2119.5 ± 144.0 μmol trolox equivalent/g, respectively. In the investigated range of concentration (10μg/ml), the aqueous acetone fractions of stem determined the highest value of 2119.5 ± 144.0 μmol trolox equivalent/g; while very least activity was generated by the petroleum ether extract of leaf (47.2 ± 5.8 μmol trolox equivalent/g), respectively. Furthermore, it can be concluded that the aqueous acetone extracts of *B.tomentosa* L. were found to be the fast and effective scavengers of ABTS radicals, as they were able to quench ABTS\(^{•+}\) radicals more readily than their respective solvent groups. The antioxidant hierarchy for aqueous acetone extract observed in the TEAC system is as follows: stem>leaf > root. Overall, the TEAC values of ethyl acetate and aqueous methanolic fractions were considerably higher than petroleum ether extracts but aqueous acetone was noted to produce maximum radical quenching effects.

![Fig. 5: Lipid peroxidation preventive property of *B.tomentosa* L. plant part extracts in β-carotene-linoleic acid system.](image-url)
Values were presented as the mean ± standard deviation of three independent experiments. Vertical bars labeled with different letters are significantly different ($P < 0.01$). Refer Table 1 for abbreviation expansions.

The antioxidant activities of the extracts were determined using β-carotene-linoleic acid coupled oxidation model system. As shown in Fig. 5, the addition of organic extracts (100 μg/ml) of *B. tomentosa* L. were found to be markedly effective in inhibiting the oxidation of linoleic acid and subsequent bleaching of β-carotene to various degrees (29.6 to 96.5%). Apparently, the aqueous acetone extracts of *B. tomentosa* L. leaf recorded the highest antioxidative power of 96.5%, respectively. Interestingly, it surpassed the performance of all the widely used natural and synthetic antioxidants tested and thereby strengthens their antioxidant power. Moreover the aqueous acetone extracts of stem and root also provided favorable antioxidant activity (75.6 and 73.9%) and their results were higher than the synthetic antioxidants and nearly equal to the natural antioxidants tested. These results postulated that the polyphenolic components might have acted as an effective antioxidant in the β-carotene-linoleic acid model system. Generally, at the specified experimental concentration (100 μg/ml) petroleum ether, ethyl acetate and methanol extracts of leaf depicted a significantly ($P<0.05$) very low value. Flavonoids such as quercetin, epicatechins, and procyanidine oligomers and other phenolic constituents of vegetables, fruits, and medicinal plants were reported as potent antioxidants in the beta-carotene-linoleic acid bleaching system (Lu and Foo, 2000). Therefore, it could be postulated that polyphenolic phytoconstituents present in the studied plant samples could be responsible for the attenuation of free radical formation during beta-carotene-linoleic acid bleaching system.

Lipid oxidation of cow blood erythrocyte membrane mediated by H$_2$O$_2$ induces membrane damage and subsequently results in hemolysis. 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 (Djeridane et al., 2006). The protective effect of different parts of *B. tomentosa* L. against H$_2$O$_2$ mediated hemolysis was investigated and presented in Fig 6. In general, all the extracts of *B. tomentosa* L. efficiently inhibited the hemolysis of erythrocytes at the concentration of 500μg/ml in the final reaction mixture and in most cases their results overwhelmed the performance of natural and synthetic antioxidants used (Fig.6). Furthermore, the aqueous acetone extracts of leaf (86%) offered more protection against erythrocyte hemolysis when compared with the other studied components, while very
least efficiency in retarding radical-induced red blood cell hemolysis was observed for non–polar solvent extracts of stem and root respectively. The anti-hemolytic activity of various extracts and standards are in the order of LA> SM> LE> Quercetin> Rutin> RA> SA> RM> LM> LP=BHA> BHT> RE> RP> SE> SP, respectively. Chaudhuri et al,(2007) reported that when red blood cells bind with flavonoids and their derivatives, the damage to the RBC membrane caused by lipid peroxidation is significantly lowered with an increase in the integrity of the membrane structure against lysis. From the results, it may be speculated that the phytochemicals present in the plant samples makes them a potent antioxidant sources which are competent in both reducing and oxidizing the free radicals. This attribute suggest that the plant samples could protect the cell membrane from lysis.

Fig. 6: Antihemolytic property of B. tomentosa L. plant parts extracted with various solvent systems.

Values were presented as the mean ± standard deviation of three independent experiments. Vertical bars labeled with different letters are significantly different (P < 0.01). Refer Table 1 for abbreviation expansion.

CONCLUSION
In conclusion, the results of the present analyze showed that leaf extract of Bauhinia tomentosa L. possesed higher and effective antioxidant activity than the related parts. The observed in vitro activities suggest that B. tomentosa leaf merit further investigation in in vivo studies against oxidative and free-radical injuries occurring in different pathological conditions. In addition, diligent research investigations on the untapped bioactive components
of the plant are urgently needed. An assessment of the toxicity and function of these extracts in food systems is also required for a better understanding of how to implement them into functional food substances and nutraceuticals.

REFERENCES


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