Antioxidant activity of the water extracts of leaves, root barks, barks of Casuarina littorea

Nusrat J. Brist, Mohammad F. Islam, Sharif M. Anisuzzaman and Mohammad N. Alam

Department of Pharmacy, Faculty of Biological Science, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

ABSTRACT

Background: Oxidation reactions in human body can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Recently, there has been an upsurge of interest in estimating the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Well known and traditionally used natural antioxidants are already being exploited commercially either as antioxidant additives or a nutritional supplement. Objective: This study has been undertaken to identify antioxidant potential of the aqueous extracts of bark, leaf and root bark of the plant Casuarina littorea. Results: Crude aqueous extracts of leaves, stem barks and root barks of the plant were prepared and evaluated for antioxidant activity. All the prepared extracts possess antioxidant potential. Based on DPPH scavenging activity, the root bark extract was the most effective one with IC_{50} 36.35 μg/mL, followed by bark and leaf extracts with IC_{50} 82.88 and 4050.34 μg/mL, respectively. Total antioxidant capacity, expressed as ascorbic acid equivalents per gram of plant extract was in the following order: root bark > bark > leaf extract. Gallic acid equivalent phenolic compounds content as well as quercetin equivalent flavonoids content were highest in the root bark extract of the C. littorea and that could be the reason behind the highest antioxidant activity of root bark extract. Conclusion: As root bark extract showed the highest antioxidant activity among all extracts, it might be investigated further for isolation of antioxidant principles.

© 2014 AENSI Publisher All rights reserved.


INTRODUCTION

A paradox in metabolism is that while the vast majority of complex life on earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species (ROS) (Davies, 1995). ROS include various free radicals such as superoxide anion (O_{2}^{-}), hydroxyl radical (•OH), as well as non-radical molecules like hydrogen peroxide (H_{2}O_{2}), singlet oxygen (‘O_{2}), and so forth. Sequential reduction of molecular oxygen (O_{2}) by high-energy exposure or electron-transfer reactions leads to formation of the highly reactive ROS. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids. In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell (Sies, 1997; Vertuani et al., 2004). However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level (Rhee, 2006). Although the body possesses defense mechanisms, as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS (Halliwell, 1995), continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage (Tseng et al., 1997). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares et al., 1997). Antioxidants of both natural and synthetic origin, able to scavenge free radicals and inhibit oxidation processes (Hayat et al., 2010). Currently, the possible toxicity (liver damage, carcinogenesis etc.) of synthetic antioxidants during industrial processing and low solubility problem has been criticized and limits the use of synthetic antioxidants (Barlow, 1990). There is an increasing interest in natural antioxidants, e.g., vitamin C, vitamin E, carotenes, xanthophylls, tannis, polyphenols, flavonoids, quinines coumarins, lignans, stilbens, alkaloids, amines betalins and carotenoids present in fruits, vegetables, medicinal
and dietary plants (Cai et al., 2003; Zheng and Wang, 2001; Anagnostopoulou et al., 2006; Silva et al., 2005; Jayaprakash and Rao, 2000). Recent researches with important bioactive compounds in many plant and food materials have received much attention. It is generally assumed that frequent consumption of plant-derived phytochemicals from vegetables, fruit, tea, and herbs may contribute to shift the balance toward an adequate antioxidant status (Halliwell, 1996). Natural antioxidants work by preventing the formation of new free radical species, converting existing free radicals into less harmful molecules and inhibiting radical-chained reactions (Rodriguez et al., 2007). For example, phenolic compounds such as, quercetin and ellagic acid, are good antioxidants that able to shield body cells from injuries mediated by reactive oxygen and nitrogen species (Sroka and Cisowski, 2003). They also exhibit a wide range of biological activity, antimicrobial activity, anticarcinogenicity and antiproliferation, and many biological activities can be attributed to their antioxidant properties (Ren et al., 2003; Tapiero et al., 2002). In this respect, phytochemicals have been shown to possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of degenerative diseases in human (Javanmardi et al., 2003).

Casuarina littorea L. (Casuarinaceae) also known as Casuarina equisetifolia L. Casuarina littorea L is commonly known as ‘JHAU’ in Bangladesh. It is a deciduous dicot angiosperm tree that superficially resembles a conifer (Division Pinophyta) (Snyder, 1992; Swearingen, 1997). The plant has been used as astringent, diuretic, emmenagogue, emmenagogue, laxative, and tonic, beefwood is a remedy for beri-beri, colic, cough, diarrhea, dysentery, headache, nerves, pimples, sores, sorethroat, stomachach, swellings, and toothache (Duke and Wain, 1981). In Malaku islands of eastern Indonesia (Moluccus), the seeds are used for passing blood in diarrhea (Burkill, 1966). Different parts of the plant such as bark, leaves, seeds and fruits have been reported to possess antihistaminic, antioxidant, antimicrobial, hepatoprotective and analgesic activities (Aher et al., 2009; Parekh and Chanda, 2007; Ahsan et al., 2009; Aher et al., 2010). An attempt has been taken in this study to evaluate its pharmacological action by means of determining the antioxidant activity of the water extracts of the leaves, root barks and barks of Causarina littorea.

**MATERIALS AND METHODS**

**Plant Material:**

The bark, leaf and root bark of the C. littorea were collected from the botanical garden of Jahangirnagar University, savar, Dhaka, Bangladesh in the month of November, 2011. The plant was identified from Laboratory of Taxonomy, Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh, DACB Accession Number 38397.

**Extraction:**

The barks, leaves, and root barks of the plants were collected in fresh condition. It was sun-dried and then, dried in an oven at reduced temperature (not more than 50°C) to make suitable for grinding purpose. Each dried part was grounded to a powder separately using a grinder. The powdered plant materials were submerged into water in an air-tight flat bottomed container for three days, with occasional shaking and stirring. The major portion of the extractable compounds of the plant materials were dissolved in the solvent. The extracts were then filtered and dried on an electrical water bath. The dried extracts were stored in respective air tight vials in a freezer until further use.

**Drugs and Chemicals:**

1, 1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin, Gallic acid and Folin-Ciocalteu reagent (FCR) was used in this investigation. All other chemicals and reagents were of analytical grade.

**Phytochemical Tests:**

Various phytochemical tests that include Molisch’s test for carbohydrates, general test for glucosides, test for glycosides, Borntragers’s test for anthraquinone glycosides, Mayer’s reagent; Hager’s reagent; Wagner’s reagent and Dragendorff’s reagent for alkaloids, Frothing test for saponins, Hydrochloric acid test for flavanoids, Salkowski’s test for steroids and Ferric chloride test for tannins.

**Determination of Total Phenol:**

The content of total phenolic compounds in plant water extracts were determined by Folin–Ciocalteu Reagent (FCR) (Folin and Ciocalteu, 1927). The FCR actually measures a sample’s reducing capacity. 1.0 mL of each plant extracts or standard of different concentration solution were taken in test tubes and 5 mL of Folin-cioalcuteu (Diluted 10 fold) reagent solution was added to the test tubes. 4 mL of Sodium carbonate solution was added into the test tubes. The test tubes were incubated for 30 minutes at 20°C to complete the reaction. (Only for standard). The test tube was incubated for 1 hour at 20°C to complete the reaction (Only for extract). The absorbances of the solutions were measured at 765 nm using a spectrophotometer against blank. The Total
content of phenolic compounds in plant methanol extracts in Gallic acid equivalents (GAE) were calculated by the following formula equation

\[ C = \frac{c \times V}{m} \]

Where:
- \( C \) = total content of phenolic compounds, mg/g plant extract, in GAE;
- \( c \) = the concentration of Gallic acid established from the calibration curve, mg/mL;
- \( V \) = the volume of extract, mL;
- \( m \) = the weight of pure plant methanol extract, g.

**Determination of Flavonoid content:**

Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al., 2002). 1 mL of sample was mixed with 3 mL of methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The concentration of flavonoids was expressed in terms of mg/100mL of sample.

**Determination of Total Antioxidant Capacity:**

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol, and carotenoids. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. (Prieto et al., 1999). Sample extracts (0.3 mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against blank. Total antioxidant capacity of the extract was measured from the regression equation prepared from the concentration versus optical density of ascorbic acid.

**DPPH Scavenging Activity:**

DPPH scavenging activity of the \( C. \) littorea was measured by the method developed by Manzocco et al. (1998). The sample extract (0.2 mL) was diluted with methanol and 2 mL of DPPH solution (0.5 mM) was added. After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated from the measured absorbance data. Ascorbic acid was used as a reference or standard antioxidant in this assay method. The percentage (%) inhibition activity was calculated from the following equation

\[ \left( A_0 - A_1 \right) / A_0 \times 100 \]

Where,
- \( A_0 \) is the absorbance of the control, and
- \( A_1 \) is the absorbance of the extract/standard.

Then % inhibitions were plotted against log concentration and from the graph IC\textsubscript{50} was calculated.

**RESULTS AND DISCUSSION**

**Preliminary Phytochemical Screening:**

The preliminary phytochemical screening study of the water extract of leaf, bark and root bark of the plant \( C. \) littorea was done to appraise the presence of bioactive components. The presence of alkaloids (Mayer, Hager, Dragnetoff, and Wagner), carbohydrates, Cardiac glycosides, steroids, saponins, tannins, flavonoids was determined. Presence of bioactive components is also confirmed by the preliminary phytochemical examination of the present study and other similar studies which are summarized in the table 1.
Table 1: Phytochemical screening of *C. littorea*.

<table>
<thead>
<tr>
<th>Extracts of plant parts</th>
<th>Alkaloids</th>
<th>Carbohydrates</th>
<th>Cardiac glycosides</th>
<th>Steroids</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MR</td>
<td>HR</td>
<td>DR</td>
<td>WR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water extract of bark</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Water extract of leaf</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water extract of root bark</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Acetone &amp; methanol extract of leaf, stem &amp; fruit</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>(Vaghasiya et al., 2011)</td>
</tr>
<tr>
<td>Methanol extract of bark</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

MR: Mayer’s reagent; DR: Dragondroff’s reagent; HR: Hager’s reagent; WR: Wagner’s reagent. (-): Absence; (+): Presence.

Total Phenolic Compound Assay:

Total phenol contents of the water extracts of bark, leaf and root bark of the plant *C. littorea* were determined by using the Folin-Ciocalteu reagent and were expressed as gallic acid equivalents (GAE) per gram of plant extract. Water extract of bark showed the highest amount of phenolic contents among the three extracts. Total Phenol content of bark and root bark was found to be similar but the leaf extract possess almost one half of the total phenolics compared to others (Table 2).

Table 2: Gallic acid equivalent (GAE) phenolic compounds content in the extracts of different parts of *C. littorea*.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Total phenols mg/g plant extract (in GAE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark</td>
<td>6.58 ± 0.03</td>
</tr>
<tr>
<td>Leaf</td>
<td>3.67 ± .30</td>
</tr>
<tr>
<td>Root bark</td>
<td>6.68 ± .03</td>
</tr>
</tbody>
</table>

Shang-Ju Zhang *et al.* (Shang-Ju Zhang *et al.*, 2010) also determined the total phenolic compound of stem bark and fine root of *C. littorea* where they used tannic acid as standards and the extracted the plant parts through with 7:3 (v/v) acetone/water solutions at room temperature. According to their study total phenolic contents in the stem bark and fine root of *C. littorea* was 110.83 ± 3.65 mg/g and 106.23 ± 11.28 mg/g, respectively (Shang-Ju Zhang *et al.*, 2010). As of our revelation, this so much differences in the result of us with them might be due to the different standard compounds. Moreover, extraction procedure of this study was also different from the earlier study.

In view of the fact that, Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi *et al*., 1992). These compounds also play a role in plant defensive mechanisms by counteracting reactive oxygen species (ROS), thus minimizing molecular damage due to microorganisms, insects and herbivores (Vaya *et al*., 1997). Phenolic compounds are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing...
peroxides (Osawa, 1994). The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart diseases and cancer is also raising interest among scientists, food manufacturers and consumers (Loliger, 1991). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g was daily ingested from a diet rich in fruits and vegetables (Tanaka et al., 1988).

**Flavonoid Content Assay:**

Total flavonoid contents of the water extracts of bark, leaf and root bark of the plant *C.littorea* was determined by Aluminium chloride colorimetric method. The total flavonoid content was calculated by using the standard curve of quercetin ($y = 0.009x - 0.036$, $R^2 = 0.972$) and was expressed as quercetin equivalents (QE) per gram of the plant extract (Table 3).

**Table 3:** Total Flavonoid Contents of the water extracts of bark, leaf and root bark of the plant *Casuarina littorea*.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total flavonoids mg/g plant extract (in QE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract of bark</td>
<td>0.234 ± .004</td>
</tr>
<tr>
<td>Water extract of leaf</td>
<td>0.118 ± .001</td>
</tr>
<tr>
<td>Water extract of root bark</td>
<td>0.936 ± .001</td>
</tr>
</tbody>
</table>

The order of the total flavonoid content of the present study followed the following order: root barks > barks > leaves (Table 2). Before our study, no one worked with the barks, leaves and root barks of the plant *C. littorea* to determine their flavonoid content. But, the use of other solvents for the extraction of parts of the plant *C. littorea* may be useful for the determination of flavonoids. So, the result from this study can be used as an important source for researchers to conduct further study of the determination of flavanoid Content of the plant.

**Total Antioxidant Capacity Assay:**

Total antioxidant capacity of the water extracts of bark, leaf and root bark of the plant *C.littorea* were determined by using the phosphomolybdenum method expressed as ascorbic acid equivalents (AAE) per gram of plant extract. The natural antioxidant ascorbic acid is used as a standard. The calibration curve of ascorbic acid is used to calculate the total antioxidant capacity ($y = 0.002x + 0.904$, $R^2 = 0.49$). The order of total antioxidant capacity of the three extracts showed that the water extract of root bark showed highest total antioxidant capacity (Table 3) equivalent to the ascorbic acid per gram of plant extract. The decreasing order of ascorbic acid equivalents in the studied parts of *C. littorea* was root bark > bark > leaf.

**Table 4:** Total antioxidant capacity of the water extracts of bark, leaf and root bark of the plant *C. litorea*.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total antioxidant capacity mg/g plant extract (in AAE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract of bark</td>
<td>8.96 ± 0.050</td>
</tr>
<tr>
<td>Water extract of leaf</td>
<td>7.16 ± 0.130</td>
</tr>
<tr>
<td>Water extract of root bark</td>
<td>9.88 ± 0.008</td>
</tr>
</tbody>
</table>

Folin-Ciocalteu assay or total flavonoid content assay determines only the amount of total phenol or total flavonoid respectively in the extract under investigation but does not necessarily represent the total antioxidant activity of the constituents present in the extract. Because the constituents present in the extract other than phenol and flavonoid might be responsible for the antioxidant action too. Therefore, result of total antioxidant capacity assay is more important for the determination of antioxidant property of an extract (Alam et al., 2012). The present study disclosed that the significant antioxidant activity is found in the root bark compared to other parts of *C. littorea*. The decreasing order of total antioxidant capacity was: root barks > barks > leaves. We were unable to compare our results with other studies as no research has been conducted on the plant *C.littorea*.

**DPPH Scavenging Activity:**

The relatively stable organic radical DPPH has been widely used in the determination of antioxidant activity of single compounds as well as the different plant extracts (Oyaizu, 1986). DPPH is nitrogen centered free radical that shows strong absorbance at 517 nm. Deep violet colored methanolic DPPH solution changes to yellow color in presence of DPPH radical scavengers. DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet color in the form of IC$_{50}$ values. IC$_{50}$ values of DPPH radical scavenging assay in different study is given in Table 5.
Table 5: IC\textsubscript{50} values of the different parts of \textit{C. littorea} in (DPPH) radical-scavenging assay.

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Plant part</th>
<th>IC\textsubscript{50} value (μg/mL)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Stem bark</td>
<td>82.88 ± 1.06</td>
<td>Present study</td>
</tr>
<tr>
<td>Water</td>
<td>Leaf</td>
<td>4050.34 ± 1.3</td>
<td>Present study</td>
</tr>
<tr>
<td>Water</td>
<td>Root bark</td>
<td>36.35 ± 0.31</td>
<td>Present study</td>
</tr>
<tr>
<td>7:3(v/v) acetone/water</td>
<td>Stem bark</td>
<td>101.69 ± 2.24</td>
<td>(Shang-Ju Zhang \textit{et al.}, 2010)</td>
</tr>
<tr>
<td>7:3(v/v) acetone/water</td>
<td>Fine root</td>
<td>89.32 ± 0.21</td>
<td>(Shang-Ju Zhang \textit{et al.}, 2010)</td>
</tr>
<tr>
<td>------</td>
<td>Ascorbic acid</td>
<td>19.89 ± 0.29</td>
<td>Present study</td>
</tr>
</tbody>
</table>

**Fig. 1:** DPPH scavenging activity of the extracts of different parts of \textit{C. littorea}.

Figure 1 shows the amount of each extract needed for 50% inhibition (IC\textsubscript{50}) or scavenging of DPPH free radical. A lower value of IC50 indicates greater antioxidant activity. The highest DPPH scavenging activity was showed by the root bark extract with IC\textsubscript{50} value of 36.35 μg/mL.

**Conclusion:**

The different parts of \textit{C. littorea} have been used to treat a variety of diseases in Bangladesh as folk medicine. Compared to the effects of leaf, root and stem barks on different diseases, little is known about the antioxidant activities of aqueous extract of different parts of \textit{C. littorea}. Our results clearly showed that all the prepared extracts possess antioxidant potential but the root bark extract had strong DPPH radical scavenging activities compared to stem bark and leaves. Moreover, the total antioxidant capacity and the phenolic contents of root bark were found higher than that of other extractives. The antioxidant activity of the plant might result from its high contents of polyphenolic compounds. Fractionation of the root bark extract with other solvent systems in further study will lead to discover the compounds responsible for the activity. So, root bark extract should be further analysed, regarding their chemical and technological aspects, such as the identification of polyphenolic and other structures responsible for activity, extractability of compounds, and stability of the active constituents. Further analyses regarding its biological properties, such as preventive capacity against LDL oxidation, bioavailability, and toxicity of the crude extract should be performed as well.

**REFERENCES**


Prieto, P., M. Pineda and M. Aguilar, 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry, 269: 337-341.


