Letter to the Editor

Evaluation of antioxidant potential of Celosia argentea extracts

Sir,

Free radicals are generated naturally required for body's basic metabolism or chemically due to malfunctioning in the antioxidant enzymes systems leading to cellular damage by the excess unstable reactive oxygen species (ROS) or reactive nitrogen species (RNS).

These ROS are free radicals with unpaired electrons (such as O₂ and H₂O₂) that are highly reactive and can react with biologic macromolecules, modify the structure and function of proteins, and cause oxidative damage to DNA through oxidative stress-induced destruction of pyrimidine and purine bases and oxidation of protein thiols and lipids. They are responsible for the disorders like arthritis, hemorrhagic shock and coronary diseases, cataract, cancer and AIDS as well as neuro-degeneration.

An antioxidant may be defined as 'any substance which when present at low concentrations, compared with those of the oxidizable substrate significantly delays or inhibits oxidation of that substrate.' These ROS are free radicals with unpaired electrons (such as O₂ and H₂O₂) that are highly reactive and can react with biologic macromolecules, modify the structure and function of proteins, and cause oxidative damage to DNA through oxidative stress-induced destruction of pyrimidine and purine bases and oxidation of protein thiols and lipids. They are responsible for the disorders like arthritis, hemorrhagic shock and coronary diseases, cataract, cancer and AIDS as well as neuro-degeneration.

Phenols contain one or more hydroxyl/carboxyl substituents which help them to chelate metal ions and thus block the superoxide-driven Fenton reaction, believed to be the most important pathway for generation of ROS. They can also suppress the lipid per-oxidation by trapping the lipid alkoxyl radical.

In the present work, Celosia argentea reported to contain high amount of plant phenolics was evaluated for its ability to scavenge the harmful radicals generated by H₂O₂, DPPH, ABTS and FRAP.

Fresh whole plant of C. argentea Linn. was collected from Bhor, Pune, Maharashtra, authenticated by Botanical Survey of India, (BSI/WC/Tech/2011), powdered and extracted successively with n-hexane, ethyl ether followed by methanol (80%).

Total Phenolic Content (TPC) was determined by mixing 100 μl of extract with 0.5 ml Folin-Ciocalteu (FC) reagent (diluted 10 times with distilled water). 7 ml of distilled water was added and allowed to stand at room temperature for 5 min. Then, 1.5 ml sodium bicarbonate (60 mg/ml) solution was added to the mixture and left in the dark at 25 °C for 30 min. The absorbance of the mixture was measured at 517 nm. BHT (25, 50, 75, 100 μg/ml) was used as reference.

%Radical scavenging activity = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} × 100

In the DPPH scavenging activity the stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Plant extracts (1 ml) (25, 50, 75, 100 μg/ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer compared with that of BHT (25, 50, 75, 100 μg/ml). In the FRAP (Ferric Reducing Antioxidant Potential) Assay the stock solutions included 300 mM acetate buffer (3.1 g sodium acetate and 16 ml acetic acid), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCI, and 20 mM FeCl₃ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml FeCl₃. The temperature of the solution was raised to 37 °C before using. Plant extracts (150 μl) (50 μg/ml) were allowed to react with 2850 μl of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 μM Trolox. The FRAP scavenging capacity of the extract was compared with that of BHT (50 μg/ml) and the percentage inhibition was calculated.

The seed extract was found to be the richest in Total Phenolic content (23.39 μg/ml) of all the three extracts at the concentration of 0.1 mg/ml. In the DPPH scavenging activity the seed extract at the concentration of 0.8 mg/ml followed by aerial parts extract. Root extract showed insignificant results when compared with the standard-ascorbic acid (97.63%).

The % inhibition of DPPH radical was highest for the seed extract at the concentration of 100 μg/ml followed by aerial parts extract.

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Root extract compared showed insignificant results compared with the standard-BHT showing % Inhibition of 99.51% at the same concentration. Thus indicating highest antioxidant activity for the seed extract compared with the standard-BHT.

The % Inhibition of ABTS radical was highest for the seed extract at the concentration of 100 mg/ml followed by aerial parts extract. Root extract, compared with the standard-BHT showed insignificant results. Thus indicating highest antioxidant activity for the seed extract compared with the standard-BHT (Table 1)(Fig. 1).

In the FRAP assay, the lower the TEAC value more is the antioxidant potential, the TEAC was found to be least for the seed extract (1.92), followed by the aerial parts extract (2.14), at the concentration of 50 µg/ml. Thus proving the seed extract to be the most potent antioxidant.

Since the plant is reported to be rich in phenols, the total phenolic for the seed extract was significant compared for all the three extracts. As per the in vitro antioxidant assays the ability to scavenge the generated harmful radicals was more for the seed extract followed by the aerial parts extract. However the antioxidant potential for the root extract was found to be negligible.

Hence, it could be stated that seed extract of C. argentea could help protect the damage due to harmful free radicals by scavenging and suppressing them possibly be due to its abundant polyphenols.

Acknowledgments

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References


Table 1

<table>
<thead>
<tr>
<th>Test extracts</th>
<th>% Inhibition H2O2 assay 0.8 mg/ml</th>
<th>% Inhibition DPPH assay 100 µg/ml</th>
<th>% Inhibition ABTS assay 100 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial part</td>
<td>67.13</td>
<td>70.81</td>
<td>62.25</td>
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<tr>
<td>Seed</td>
<td>79.12</td>
<td>88.18</td>
<td>86.05</td>
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<tr>
<td>Root</td>
<td>51.14</td>
<td>36.16</td>
<td>30.80</td>
</tr>
<tr>
<td>Standard</td>
<td>97.63</td>
<td>99.51</td>
<td>87.76</td>
</tr>
</tbody>
</table>

Std for H2O2 Assay: Ascorbic acid.
Std for DPPH & ABTS Assay: BHT.

Fig. 1. % Inhibition of various extracts at the highest concentration compared to standard.