ABSTRACT

Herbs have been used as a dietary source of nutrition and play a vital role in improving our health. Various metabolic activities in our body result in the formation of the free radicals or reactive oxygen species (ROS) that leads to the onset of many diseases such as cancer, rheumatoid arthritis, liver diseases and atherosclerosis as well as in degenerative processes associated with ageing. Antioxidant compounds in diet play an important role as a health protecting factor. The present study was aimed at investigating the phytochemical constituents and antioxidant activity of Oldenlandia corymbosa having ethnomedicinal property. Free radical scavenging activity was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The methanolic extract exhibited antioxidant activity significantly in the plant extracts. The IC$_{50}$ value of the methanolic extract was found to be 129.28 ± 1.59µg/ml. The study has revealed that the extracts of this plant can be used as therapeutic agent as it would exert several beneficial effects by virtue of their antioxidant activity and also can be a rich source of nutrition in our diet system as it was rich in its phytochemical constituents.

Keywords: Oldenlandia corymbosa, Antioxidant Activity, DPPH, Total phenolic content, Ascorbic acid, Gallic acid.

INTRODUCTION

Dietary habits of population in different region of the world have been determined mainly by the availability of foods locally and local practices. Though satisfaction of hunger is the...
primary criteria of diet, for sustaining healthy and active life, diet should contain proper nutritional elements. Generally, nutrients are those substances which have health promoting as well as health protective activity. Broadly nutrients can be divided into two types- macro nutrients and micro nutrients. Macro nutrient includes protein, carbohydrate, lipid or fat and crude fibre. On the other hand, micro nutrient includes different minerals, vitamins, antioxidants etc. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. During the metabolic process reactive oxygen species (ROS) is derived which involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with ageing [1]. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione [2]. When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur, resulting in diseases and accelerating aging. However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies reduce oxidative damage. It has been reported that the antioxidant activity of plant materials are well correlated with the content of their phenolic compounds [3,4]. Phenolic compounds, especially phenolic acids and flavonoids, are ubiquitously present in vegetables, fruits, seeds, tea, wines and juices; thus they are an integral part of the human diet. In a broad sense, nutritive foods are those foods, which not only provide nutrition but at the same time have health protective and health promoting properties. In India, many vegetables, fruits and plant based foods are known to have such health promoting and protective properties.

*Oldenlandia corymbosa* Linn. is a flowering plant in the family Rubiaceae that grows throughout India and other tropical regions of the world. It is found widely in the Brahmaputra Valley region of the North Eastern part of India. The present study was made to evaluate the nutraceutical properties and antioxidant activity of these plants.

**MATERIALS AND METHODS**

The whole plants of *Oldenlandia corymbosa* were collected from the foot hills of Nilachal, Guwahati, Assam. Collected herbs are washed thoroughly, sliced and oven dried at 60°C until we get constant weight. The dried slices were powdered and kept at 4°C for further analysis.
Methods of analysis: Chemical analysis is done on moisture free basis. Analysis is carried out to estimate the macro nutrient components viz. total protein, total carbohydrates and ascorbic acid and antioxidant activity of the samples.

Total Protein Estimation: The total protein content of Oldenlandia corymbosa is estimated by following the method developed by Lowry et. al. [5]. Extraction is carried out with buffers used for the enzyme assay. 500 mg of the sample is grinded well with a mortar and pestle in 5ml of the buffer and after centrifuging; the supernatant is used for protein estimation. The reading is taken in a UV-Vis Spectrophotometer at 660nm and the amount of protein present is calculated by plotting the value in a standard curve of Bovine Serum Albumin (BSA).

Total Carbohydrate Estimation: The total carbohydrate content of the samples is estimated by Anthrone method [6]. 100 mg dried samples were hydrolyzed with 2.5 N HCl for about 3 hours in a boiling water bath. Sodium carbonate was added to neutralize the extracts. Subsequently the extracts were centrifuged and supernatant were collected. The residue was washed thrice with distilled water and all the supernatants were pooled and final volume was adjusted to 100ml. 0.5 ml of the extracts were taken and volume made up to 1ml distilled water. 4ml of Anthrone reagent was added to the above solution. Absorbance was taken in a UV-Vis spectrophotometer at 630 nm and the amount of carbohydrate present was calculated by plotting the value in a standard curve of Standard Glucose solution.

Crude Fibre Content: Crude fibre in the samples was determined by the method described by Maynard [7]. Defatted sample (2g) was placed in a glass crucible and attached to the extraction unit. 150 ml boiling 1.25% sulphuric acid solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150 ml) was added. The sample was digested for 30 min, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in a desiccator and weighed (W1). The sample was then ashed at 600°C in a muffle furnace (for 2 h, cooled in a desiccator and reweighed (W2). Extracted fibre was expressed as percentage of the original undefatted sample and calculated according to the formula:

\[
\text{% crude fiber in ground sample} = \frac{\text{Loss in weight on ignition (W2 - W1)} - (\text{W3 - W1})}{\text{Weight of the sample}} \times 100
\]
Ascorbic Acid Content Estimation: The amount of ascorbic acid present in the sample was calculated by extracting the sample in 4% oxalic acid and titrating the extract against the 2,6-dichloro phenol indophenols dye until the end point where pink colour appears that persist for a few minutes\(^8\). The amount of dye consumed is equivalent to the amount of ascorbic acid present in the samples. Standard ascorbic acid solution is used as the reference and the calculation is done by the following formula:

\[
\text{Amount of ascorbic acid (mg/100gm) sample} = \frac{0.5 \text{mg} \times V_2 \text{ml} \times 100 \text{ml}}{V_1 \text{ ml} \times 5 \text{ml} \times \text{Wt. of the sample}} \times 100
\]

Where, \( V_1 \) = volume of oxalic acid, \( V_2 \) = volume of the sample

Total Phenol Content Estimation: The total phenol content was determined by the Folin-Ciocalteau’s method\(^9\). 200\(\mu\)l of the herb extracts (1mg/ml) was taken and volume made up to 2ml. 0.3 ml of Folin-Ciocalteau reagent was added. After 5mins, 0.8 ml of 20% \( \text{Na}_2\text{CO}_3 \) was added and the final volume was made 5ml. Absorbance was taken by UV-Vis Spectrophotometer at 765nm after 30 minutes incubation. The amount of phenol content was determined using gallic acid as standard. Results were expressed as \( \mu \text{g}/\text{mg} \) (Gallic acid equivalent/dry weight) and the calculations were done by using the following formula:

\[
\text{TPC} = C \times \frac{V}{m}
\]

Where, TPC = total phenol content, \( C \) = concentration of Gallic acid (mg/ml), \( V \) = volume of plant extract (ml) and \( m \) = weight of pure plant extract (g)

Antioxidant activity estimation: The antioxidant activities of the herb extract along with standard were assessed on the basis of the radical scavenging effect of stable DPPH\(^{10}\). A solution of DPPH of concentration 0.2 mM was prepared in 70% methanol and kept overnight. Stock solution (1mg/ml) of the extract was prepared in 70% methanol. Various concentration of the extracts \( \text{viz.} 10, 20, 50, 100, 150, 200, 300, 400 \) and 500 \( \mu \)l were taken in different test tubes and the volume was made up to 1000 \( \mu \)l. 1 ml DPPH was added to each solution and kept at dark for 30 minutes. Ascorbic acid and Gallic acid were taken as standards. Optical density of these samples was measured at 517 nm along with blank where 1ml methanol with 1 ml DPPH solution was taken. The activities of the samples are measured in terms of percent inhibition (IC\(_{50}\)) and calculated by the following formulae:

\[
\text{Percent (\%) inhibition of DPPH activity} = \frac{A - B}{A} \times 100
\]
Where,  
\[ A = \text{Optical density of the blank} \]
\[ B = \text{Optical density of the sample} \]

**Statistical Analysis:** The data were subjected to statistical analysis. All the assays were recorded in triplicates and the values were expressed as mean ±S.D. IC\(_{50}\) value was calculated by plotting a graph with percent inhibition on y-axis and concentration on x-axis.

**RESULT AND DISCUSSION**

Table 1: Phytochemical analysis of *Oldenlandia corymbosa.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbohydrate Content (%)</th>
<th>Protein Content (%)</th>
<th>Crude fibre (%)</th>
<th>Ash content (gm)</th>
<th>Ascorbic Acid Content (mg/100g)</th>
<th>Total Phenol Content (µgGAE/mg)</th>
<th>Inhibition Concentration (IC(_{50})) (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oldenlandia corymbosa</em></td>
<td>29.53 ± 0.15</td>
<td>3.65 ± 0.18</td>
<td>23.90 ± 0.14</td>
<td>0.09 ± 0.15</td>
<td>47.5 ± 1.09</td>
<td>32.68 ± 0.39</td>
<td>129.28 ± 1.59</td>
</tr>
</tbody>
</table>

*Values represented in the table are mean ±S.D of three replicates.

Figure 1: DPPH free radical scavenging activity of methanolic extract of *Oldenlandia corymbosa* at 517nm.

The phytochemical analysis was done for total carbohydrate content, total protein content, total ascorbic acid content and total phenol content of *Oldenlandia corymbosa*. The antioxidant activity was measured using DPPH assay. In *Oldenlandia corymbosa*, the total phenol content was found to be 32.68 ± 0.39 µgGAE/mg whereas the ascorbic acid content was 47.5 ± 1.09 mg/100 gm. The carbohydrate content was found to be 29.53 ± 0.15% and
the protein content was 3.65 ± 0.18 %. The antioxidant activity of *Oldenlandia corymbosa* was found to be 129.28±1.59 µg/ml.

The most common antioxidants present in herbs are vitamins C and E, carotenoids, flavonoids and thiol (SH) compounds, etc. There were several reports that highlights the contribution of phenolic compounds and ascorbic acid to antioxidant activity \[11,12,13\]. The present investigation suggests that the major source of antioxidant capacity of *Oldenlandia corymbosa* is both ascorbic acid and phenolic compounds. The protection in the body provided against oxidative damage by fruit and vegetables has been attributed to the fact that these foods may provide an optimal mix of phytochemicals, such as natural antioxidants and other bioactive compounds. Therefore, the supplementation of these natural antioxidants through a balanced diet containing adequate herbs could be much more effective than the supplementation of an individual antioxidant such as vitamin C or vitamin E.

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**REFERENCE**


