**ABSTRACT**

Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action. The present study involves in screening of the phytochemical and antioxidant evaluation of Petroleum ether, chloroform and methanolic extracts of the leaves of *Alternanthera sessilis*, *Tephrosia purpurea* and *Clitoria ternata* with a view to enumerating the potential areas of their utilization in the chemical and pharmaceutical industries. Our results were compared with a standard antioxidant, BHT. Results clearly showed that *Clitoria ternata*, *Tephrosia purpurea* possess high phytochemical compounds and also exhibit high antioxidant activity compared to *Alternanthera sessilis*.

**Key words:** Phytochemicals, Total phenolics, Total Flavonoids, Total antioxidant power, DPPH assay.

**INTRODUCTION**

Medicinal plants contain substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Sofowora, 1982). In present era, herbal medicine, or drugs from medicinal plants are used in the treatment and cure of sicknesses and diseases conditions. It is therefore important that we continually evaluate and develop our indigenous plant genetic resources for the improvement and sustenance of our health care delivery system. Hence, knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agents. Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants (Erturk et al., 2006; Mohanta et al., 2007). The most important bioactive phytochemical constituents including alkaloids, tannins, flavonoids and phenolic compounds (Kumar et al., 2007).

The present study involves in elucidating the phytochemical constituents and antioxidant status of petroleum ether, chloroform and methanolic extracts of the leaves of *Alternanthera sessilis*, *Tephrosia purpurea* and *Clitoria ternata*. *Alternanthera sessilis* belongs to Amaranthaceae is branched, glabrous, succulent herb and leaves are simple or pinnately compound. The plant is accredited with galactagogue properties and is a good fodder increase the flow of milk in the cattle and also used to treat night blindness. *Tephrosia purpurea* belongs to family Fabaceae is a much branched, erect, perennial herb and is antihelmintic, a herb and common garden flower plant used in the treatment of eye infections, skin diseases, urinary troubles, ulcers and has antiodotal properties.

**MATERIALS AND METHODS**

Folin-Ciocalteu reagent. Aluminum chloride was obtained from Himedia Laboratories Pvt. Ltd, Mumbai, India. Tannic acid was procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All other chemicals used were of analytical grade obtained from commercial sources.

**Preparation of the petroleum ether, chloroform and methanol extracts of the sample:**

The Leaves of above medicinal plants were collected and was dried under shade and then powered with a mechanical grinder to obtain a coarse powder, which was then subjected to successive extraction in a Soxhlet apparatus using different solvents such as petroleum ether, chloroform and methanol based on the increase in polarity. Solvent elimination under reduced pressure afforded the petroleum ether, chloroform and methanolic extracts respectively. The resulting petroleum ether, chloroform and methanol extracts were then used for the present study.

**Analysis of total phenolics**

The total phenolics were determined using the Folin Ciocalteu reagent as reported by Javanmardi et al., (2003). To 50 µl of the plant extract, 2.5ml of diluted Folin Cin-Calteu reagent and 2.0 ml of 7.5% (w/v) sodium carbonate was added and incubated at 45°C for 15 min. The absorbance values of all samples were measured in a spectrophotometer at 765 nm. A set of standard solutions of Gallic acid is treated in the same manner as described earlier and read against a blank. The results were expressed as mg of Gallic acid equivalents per gm weight.

**Total flavonoids determination**

Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al., 2002). 0.5 ml of each plant extracts were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Visible spectrophotometer. The calibration standard curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol and results were expressed as Quercetine equivalents.

**Estimation of tannins**

Standard curves for tannin were constructed using the method of Folin and Ciocalteu (1927). To 0.1 ml solution of sample solution, 6.9 ml of distilled water was added and the contents were mixed with 1.5 ml of 20% sodium carbonate and 0.5 ml of folin-reagent phenol. The mixture was shaken well, kept at room temperature for 1 h and absorbance was measured at 725 nm in a spectrophotometer. A set of standard solutions of Tannic acid is treated in the same manner as described earlier and read against a blank. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

**Estimation of total Alkaloids**

Total Alkaloid content was estimated by the method of Sreevidya and Mehrotra, 2003. A standard solution was prepared by dissolving 5mg of boldine and plant sample separately in 5 mL of warm distilled water each. Five mL of boldine solution/sample extract was adjusted to pH 2-2.5 (with 0.01 M HCl), and 2 mL of DR was added to form an orange precipitate that was centrifuged at 5000 rpm for 15 min. Afterward, DR was added to the supernatant to check for complete precipitation. A 2 mL amount of 1% sodium sulfide was added to the residue to form a brownish black precipitate which was centrifuged at 5000 rpm for 15 min. Complete precipitation was checked by further adding 1% sodium sulfide. The resulting residue was dissolved in 2 mL of nitric acid with warming and sonication and then made up to 10 mL with distilled water. A 5 mL amount of 3% thiourea was added to 1 mL of the resulting solution to form a yellow bismuth complex, of which the absorbance was measured at 435 nm. All the assays were performed in triplicate. The amount of bismuth present in the boldine solution/extract was achieved from the calibration curve of bismuth nitrate. The results were expressed as boldine, considering that is a monobasic alkaloid, and therefore the complex formed with bismuth follows a 1:1 stoichiometry.

**Antioxidant and Radical Scavenging assays**

**Total Antioxidant power/Ferric reducing ability (FRAP) assay**

The total antioxidant power of the sample was assayed by the method (Benzie and Strain, 1996). 3.0 ml of FRAP working reagent (2.5 ml of 0.1 M acetate buffer, pH 3.6, 0.25 ml of 0.3 mM 2, 4, 6-Tri pyrydyl-s-triazine (TPTZ) solution and 0.25 ml of 10 mM FeCl3.6H2O) was taken in a test tube then 100
The antioxidant activities of the present samples were determined by FRAP assay and DPPH methods and the results were compared with Butylated Hydroxytoluene (BHT), a synthetic and a well established antioxidant. The % of inhibition of DPPH radical scavenging assays was shown to be maximum in BHT (294 BE) and low in A. sessilis (64 BE). The presence of phytochemicals, in addition to vitamins and provitamins, in plants has been recently considered of crucial nutritional importance in the prevention of chronic diseases such as cancer, cardiovascular disease, and diabetes etc., (Aruoma, 2003). Because prevention is a more effective strategy than treatment for chronic diseases, a constant supply of phytochemical containing plants with desirable health benefits beyond basic nutrition is essential to furnish the defensive mechanism to reduce the risk of chronic diseases in humans (Liu RH, 2002). Many of the phytochemicals have been found to provide a much stronger antioxidant activity than vitamins within the same food (Eberhardt et al., 2000).

### Table 1: showing the levels of phytochemicals

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total Phenolics (GAE)</th>
<th>Total Flavonoids (QE)</th>
<th>Total Tannins (TA)</th>
<th>Total Alkaloids (BE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic extract</td>
<td>51.5</td>
<td>27.5</td>
<td>58.8</td>
<td>91</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>27.5</td>
<td>24.1</td>
<td>30.8</td>
<td>48</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>18.4</td>
<td>16.0</td>
<td>19.2</td>
<td>16</td>
</tr>
</tbody>
</table>

Each is a mean of triplicates.

GAE - Gallic acid Equivalents, QE - Quercetine Equivalents, TA - Tannic acid Equivalents, BE - Boldine Equivalents.

### RESULTS AND DISCUSSION

Phytochemical analysis

The results of the phytochemical studies were presented in the table.1. The results clearly showed that the higher amounts of estimated phytochemicals were found to be maximum in C. ternata followed by T. purpurea and minimum in A. sessilis. Total tannins were observed to be low in A. sessilis (52.3 mg of tannic acid/mL of extract), C. ternata (111.9 mg of tannic acid/mL of extract) and high in T. purpurea (126.6 mg of tannic acid/mL of extract). Total alkaloid content was found to be high in C. ternata (411 BE) followed by T. purpurea (294 BE) and low in A. sessilis (64 BE). The presence of phytochemicals, along with vitamins and provitamins, in plants has been recently considered of crucial nutritional importance in the prevention of chronic diseases such as cancer, cardiovascular disease, and diabetes etc., (Aruoma, 2003). Because prevention is a more effective strategy than treatment for chronic diseases, a constant supply of phytochemical containing plants with desirable health benefits beyond basic nutrition is essential to furnish the defensive mechanism to reduce the risk of chronic diseases in humans (Liu RH, 2002). Many of the phytochemicals have been found to provide a much stronger antioxidant activity than vitamins within the same food (Eberhardt et al., 2000).

In conclusion, all the extracts obtained from sequential solvent extraction were subjected to phytochemical and antioxidant analysis. It was observed that, the methanolic and chloroform extracts of C. ternata and T. purpurea possess a considerable amount of phytochemicals and were found to be effective antioxidants compared to A. sessilis. These medicinal plants could be potential rich resources of phytochemical and natural antioxidants and could be developed into functional food or drug for prevention and treatment of diseases caused by oxidative stress. In the future, the specific components with high antioxidant capacity in these medicinal plants should be isolated and identified and explored for their health effects with oxidative stress.

### REFERENCES

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**Source of support:** Nil, **Conflict of interest:** None Declared