Evaluation of *In vitro* Antioxidant Activity of Some Plants of Cachar District, Assam.

Paul S. B.¹, Mazumder A. H.²*, Gogoi H. K.¹, Gogoi B. J.¹, Chaurasia A K¹, Singh L¹, Srivastava R. B.³

¹Defence Research Laboratory, Post Bag No. 02, Tezpur, Assam, 784 001, India
²Department of Chemistry, Assam University, Silchar, Assam, 788 011, India
³Directorate of Life Sciences, Ministry of Defence, Govt. of India, DRDO HQ, New Delhi, India

* Corresponding author. Tel: +91 3712 258836 (O), Mob: +91 9435738250
E-mail address: mdafjal123@rediffmail.com (Mazumder. A. H.)

**ABSTRACT**

The present study was undertaken to evaluate *in vitro* antioxidant activity of methanolic extract from leaves of four plants viz. Clerodendron colebrookianum Walp. (Verbinaceae), Gnetum gnemon L. (Gnetaceae), Sarcochlamys pulcherrima (Roxb.) Gaud. (Urticaceae), Garcinia lancifolia (Don) Roxb. (Cluciaceae), from Cachar district, Assam, India. DPPH (1,1-diphenyl 2-picrylhydrazyl) radical scavenging capacity, reducing power assay (RPA) and photochemiluminescence (PCL) assay were used for evaluating *in vitro* antioxidant activity. Total phenolic content (TPC) was estimated by Folin–Ciocalteu’s method. Sarcochlamys pulcherrima showed highest antioxidant activity (DPPH EC<sub>50</sub> 9.70 ±1.51ppm), as compared to C. colebrookianum (121.05 ±1.09ppm), G. gnemon (255.99 ±0.82ppm), and G. lancifolia (344.96 ±0.76ppm). Highest activity of S. pulcherrima was also supported by RPA and PCL and highest TPC (0.33 mg gallic acid equivalent/mg of dry extract) amongst the plants, indicated that phenolic compound are mainly responsible for the activity.

**Keywords:** S. pulcherrima, Antioxidant activity, DPPH, PCL, RPA, TPC.

**Editor:** Srisailam Keshetti, Phcog.Net

**Copyright:** © 2010 Phcog.net

*Author for Correspondence: mdafjal123@rediffmail.com

**INTRODUCTION**

Reactive oxygen species (ROS) viz. superoxide radicals, hydroxyl radicals, and hydrogen peroxide are generated as byproducts of biological reactions during normal cell aerobic respiration. Oxidative damage may also cause great damage to cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity and DNA mutations leading to cancer, atherosclerosis, hypertension and other diseases [1]. Natural antioxidants with multifunctional potential are of high interest as alternatives for synthetic antioxidants to prevent oxidation. Northeast India is bestowed with innumerable flora of the world and Clerodendron colebrookianum, Gnetum gnemon, Sarcochlamys pulcherrima and Garcinia lancifolia are unexplored plants for antioxidant activity. Tender leaves of all plants are used as vegetables by Khasi and Naga tribe of Machikhal, Binnakandi, Ramnagar, and other tribal pockets of Cachar District, Assam. Tribal people of this area use the water decoction of leaves of C. colebrookianum to cure high blood pressure. Leaves of G. gnemon are made into paste and applied in athlete’s foot. S. pulcherrima leaves are claimed to damage tape worm egg present in pork when boiled with it. Leaf juice of G. lancifolia is taken in headache. Although, previous study showed presence of different class of phytochemicals in the leaves of C. colebrookianum [2-8], and G. gnemon [9-10], no phytochemical report was found in the case of Sarcochlamys pulcherrima and Garcinia lancifolia leaves. Moreover, *in vitro* antioxidant activities of leaves of these plants were not explored yet.

Though these tribal peoples are residing in a tough condition involving much physical labour in every aspect of survival but they are able to bear up. Intake of antioxidant is reported as a remedy for fatigue and tiredness [11-12]. We hypothesized that the antioxidant intake (unknown to them) through the consumption of these medicinal vegetable may be the reason of sound health and physical stamina of these tribal peoples. Therefore, the present investigation was undertaken to study the antioxidant potential of these herbs and to
put forward the evidence of the fact that these plants are having good antioxidant activity.

**MATERIAL AND METHODS**

**Plant material and extraction**

Leaves of four plants viz. Clerodendron colebrookianum Walp. (Verbinaceae), Gnetum gnemon L. (Gnetaceae), Sarcochlamys pulcherrima (Roxb.) Gaud. (Urticaceae), Garcinia lancifolia (Don) Roxb. (Cluciaceae) were collected from Arun Punjee (Tribal Village) of Machkhal, Cachar District, Assam, India, during the month of April and authenticated by Botanical Survey of India, Shillong, Meghalaya and voucher specimen were kept in the repository of Phytochemistry Division, Defence Research Laboratory, Tezpur for future reference. The plant material was extracted by cold maceration in mixture of methanol and water (80:20). Further, solvent was evaporated to dryness in rotary evaporator under reduced pressure and kept in refrigerator (0°C) for future uses.

**Evaluation of Antioxidant Activity**

**Radical scavenging activity by DPPH method**

Radical scavenging activity of the sample extracts were measured by colorimetric assay using DPPH as a source of free radical and according to the method of Blois.[13] Briefly, 1 ml of the crude extract solution at variable concentrations (2.5 –1000 μg/ml in methanol) was added to 1 ml of a DPPH solution at concentration 40μg/ml in methanol, kept for 35 min at room temperature until to produce stable colour and absorbance was measured at 517 nm using a SPECORD-200 UV–Vis spectrophotometer (Analytic Jena AG, Jena, Germany). Increased absorbance of the reaction mixture indicated the increased reducing power.

**Total antioxidant activity by Photochemiluminescence**

Total antioxidant activity was determined by Photochemiluminescence method using Integral antioxidative capacity of lipid soluble substances (ACL) kit in the antioxidant analyser called Photochem® (Analytic Jena AG, Jena, Germany) [15]. In this method, a photosensitizer substance in standardised volumes acts as a source of superoxide anion radical which produces the radicals by optical excitation. Residual radicals remained after partly reacting with the antioxidants present in the sample cause the detector substance luminol to luminesce. The luminescence is then determined.

**ESTIMATION OF TOTAL PHENOLIC CONTENT**

Total Phenolic content (TPC) were measured by Folin-Ciocalteu’s method [16]. Briefly, 1 ml of each sample (1000 μg/ml) was added with 25ml distilled water and 5ml of Folin-Ciocalteu’s phenol reagent followed by 15 ml of Na₂CO₃ (20%, w/v) and made up to 100ml followed by incubation at room temperature for 2 hrs. The absorbance of all samples was measured at 760 nm using a SPECORD-200 UV–vis spectrophotometer (Analytic Jena AG, Jena, Germany). Results were expressed as milligram of gallic acid equivalent per milligram of dry weight (mg GAE/mg dw).

**RESULTS AND DISCUSSION**

**Radical scavenging activity by DPPH method**

DPPH method allows estimation of hydrogen radical donating ability of the test extract. This model represents the situation in metabolic system where an antioxidant will stabilise a free radical by reacting with hydrogen radical. The results are expressed in EC₅₀ (Effective Concentration to reduce initial concentration of DPPH to 50%). Lesser the EC₅₀ value for an extract is associated with higher ability to donate hydrogen radical i.e. antioxidant activity.
activity. In the present study EC$_{50}$ values were found to be 121.05, 255.99, 9.70 and 344.96 for C. colebrookianum, G. gnemon, S. pulcherrima and G. lancifolia respectively. These data clearly indicate that S. pulcherrima is promising radical scavenger.

**Reducing power assay**

The results are expressed as absorbance X 100 and results are 14.40, 8.37, 161.8 and 7.02 for C. colebrookianum, G. gnemon, S. pulcherrima and G. lancifolia respectively. Higher absorbance represents higher reducing ability of an extract. Out of the four plants, highest absorbance was observed in the case of S. pulcherrima (161.8), which is in accordance with the result of DPPH assay.

**Total antioxidant activity by Photochemiluminescence**

This automated method allows precise determination of antioxidant activity. Trolox at different concentration (0.5–5 nmol) was run as standard, and sample was diluted so that the results lie within the concentration range of the standard. The results were calculated considering the concentration and dilution factor. S. pulcherrima showed highest activity as reflected from the result (6462.63 nmol equivalent of Trolox/mg of dry extract).

**Estimation of total phenolic content**

Phenolic compound are having wide bioactivity including antioxidant. The antioxidant activity of phenolic compound is due to hydroxyl functional group, however other factors e.g., presence of electron withdrawing or releasing group in the aromatic ring having hydroxyl moiety will increase or decrease the activity. To establish the correlation of antioxidant activity indicated by different assays with phenolic compound, total phenolic content was estimated and observed that S. pulcherrima which showed higher antioxidant activity have highest phenolic content i.e., 0.33mg GAE /mg of dry extract.

The result can be explained with previous study as presence of phenolic content is responsible for antioxidant activity [17]. S. pulcherrima leaf extract contain highest phenolic content as compared to other plants and showed maximum antioxidant activity. Correlation coefficient between DPPH EC$_{50}$ value and TPC is -0.777 but increases to -0.972, if the data of C. colebrookianum is excluded. This is due to the fact that C. colebrookianum may contain antioxidant active compound other than phenolics and hence alters the correlation. But in both the cases, there is strong correlation between DPPH EC$_{50}$ value and TPC. Earlier studies show that DPPH and TPC value have a negative correlation i.e. as the TPC value increases, DPPH EC$_{50}$ value decreases in similar fashion[18–20]. Previous studies are in agreement with present one, where S. pulcherrima showed lowest DPPH EC$_{50}$ value and highest TPC value. Result of reducing power assay and total antioxidant activity determined by using Photochem*, further supports the highest activity of S. pulcherrima and considered as most potent antioxidant plant.

**CONCLUSION**

Our study provided the clue that leaves of S. pulcherrima is having potent antioxidant activity and the activity is due to the presence of phenolic compound. The plant is consumed by Khadi and Na tribe of Cachar District, Assam and the present finding partially validate their traditional knowledge about the goodness of consumption of this medicinal plant as vegetable. Findings of the present work can provide a first hand information in developing antioxidant based antistress products. Further study is going on to isolate phytochemicals and in vivo antioxidant study to validate the in vitro antioxidant activity.

<table>
<thead>
<tr>
<th>Plant</th>
<th>DPPH EC$_{50}$†</th>
<th>PCL† (RSD %)</th>
<th>RPA‡</th>
<th>TPC¥</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. colebrookianum</td>
<td>121.05 ±1.09a</td>
<td>375.52 (0.03)a</td>
<td>14.40±2.24</td>
<td>0.0429 ± 1.92</td>
</tr>
<tr>
<td>G. gnemon</td>
<td>255.99 ±0.82</td>
<td>16.59 (2.53)</td>
<td>8.37±4.03</td>
<td>0.0452 ± 3.85</td>
</tr>
<tr>
<td>S. pulcherrima</td>
<td>9.70 ±1.51</td>
<td>6462.63 (0.84) a</td>
<td>161.8±3.10 a</td>
<td>0.33 ± 12.25 a</td>
</tr>
<tr>
<td>G. lancifolia</td>
<td>344.96 ±0.76</td>
<td>192.08 (0.39)</td>
<td>7.02±2.17</td>
<td>0.0443 ± 4.14</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ±SD (n=6), except PCL data. The significant difference was analysed by one-way Anova followed by Tukeys post hoc test. p<0.05 was considered significant. Comparison is made between G. lancifolia with other plants.
† EC$_{50}$ in μg/ml.
‡ In nmol equivalent of Trolox/mg of dry extract,
§ In mg Gallic Acid equivalent/mg of dried extract,
¥ Absorbance given by 1000ppm solution of extract × 100
ACKNOWLEDGEMENTS:

The authors are thankful to Dr. Pronobesh Chattopadhyay, Sc ‘D’, DRL, Tezpur, Assam, India, for helping in manuscript preparation & statistical analysis and Mr. Dilip Palong, Machkhal Arun Punjee for sample collection. One of the authors (Mazumder., A. H.) is thankful to Defence Research and Development Organisation (DRDO), Ministry of Defence, Government of India, for the award of Research Fellowship.

REFERENCES