Hydroxyl radical activity of *Amaranthus gangeticus* leaves.

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ABSTRACT

*Amaranthus gangeticus* is a medicinal plant. Our aim is to investigate its invitro antioxidant property. Hence 70% ethanolic extract of *Amaranthus gangeticus* leaves (AGEE) was taken and the parameter studied was hydroxyl free radical scavenging activity. *In-vitro* model was carried out to evaluate its antioxidant activity. Therefore these results concluded that, the ethanolic extract afford significant antioxidant activity which may be attributed due to polyphenols.

KEYWORDS

AGEE. Hydroxyl radical and polyphenols.
1. INTRODUCTION

*Amaranthus gangeticus* is an edible plant used as vegetable which is being used by native practitioner as hepatoprotective in treating various types of jaundice. The leaves of this plant contain polyphenolic compounds like tannins and flavonoids. These polyphenolic compounds have antioxidant property. Therefore anti-oxidants have been known to possess hepatoprotective activity. Keeping the native knowledge and the above mentioned literature information¹, this plant was selected for present study to screen the leaves of this edible plant for the presence of phytoconstituents, and antioxidant activity. This study was carried out by using AGEE as antioxidants.

2. MATERIALS AND METHODS

Collection and identification of plant: The plant was collected from Kusnoor village (Gulbarga district), in the month of March and was authenticated by Dr. Srinath Rao, chairman, P.G. Department of Studies and Research in Botany, Gulbarga University, Gulbarga, Karnataka. The plant was thoroughly cleaned and the leaves were shade dried and made into a coarse powder by rubbing in the palms.

2.1. Extraction

200 gm of shade dried leaf powder of *Amaranthus gangeticus* was extracted in Soxhlet apparatus using petroleum ether for defatting and then it was extracted with 70% ethanol. This solvent was evaporated on a water bath at a low temperature (50°C) and finally the residue was obtained.

2.2. Materials used

All chemicals and reagents used were of analytical grade.

2.3. In-vitro antioxidant activity- Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of AGEE was studied by using method of Sasanka et. al.² In biochemical systems, superoxide radical and H₂O₂ react together to form a hydroxyl radical OH•, this attacks and destroys almost all known biomolecules. When phenyl hydrazine is added to erythrocytes, it causes peroxidation of endogenous lipids and alteration of membrane fluidity. This peroxidative damage to erythrocytes is probably initiated by active oxygen species like O₂•, OH• and H₂O₂, which are generated in solution from autoxidation of phenyl hydrazine. This forms the basis of this experiment².

2.3.1. Procedure

Hydroxyl radical generation by phenyl hydrazine has been measured by the 2-deoxyribose degradation assay of Hathwell and Gutteride³. In 50 mM phosphate buffer (pH 7.4) 1 mM deoxyribose, 0.2 mM phenyl hydrazine hydrochloride were prepared. 0.6 ml of 1 mM deoxyribose and 0.4 ml of AGEE (varying doses 10, 20, 25, 50 and 100 μg) and sodium metabisulphate (25μg Std.) were taken. 0.2 ml of Phosphate buffer was added to make the volume to 1.6 ml. The reaction mixture was incubated for 10 min and 0.4 ml of 0.2mM phenyl...
Hydrazine HCl was added and incubated for 1 hr and 1 ml each of 2.8% TCA and 1% (w/v) of thiobarbituric acid were added. The reaction mixture was heated for 10 minutes on a boiling water bath. The tubes were cooled and absorbance was taken at 532nm by using UV-double beam spectrophotometer. Decrease in the absorbance is indicating the increase in the hydroxyl free radical scavenging activity. The results are compiled in Table 1.

\[
\% \text{ Radical scavenging activity} = \frac{\text{control Abs} - \text{sample Abs}}{\text{control Abs}} \times 100 \quad (1)
\]

2.4. Statistical analysis
The data presented in Table No. 1 (n=3) were expressed as mean ± SEM. Significant difference among the mean were calculated at the level of p < 0.001 and analyzed by one-way analysis of variance by Dunnet’s ‘t’ test. A value of p < 0.05 was defined as significant.

Table 1. Hydroxyl Radical scavenging activity of 70% ethanolic extract of *Amaranthus gangeticus* leaves.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Absorbance Mean ± SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.313±0.003</td>
<td>---</td>
</tr>
<tr>
<td>Control + Standard 25 µg</td>
<td>0.103±0.003***</td>
<td>67.741</td>
</tr>
<tr>
<td>Control + 70 % ethanolic extract 10µg</td>
<td>0.343±0.003***</td>
<td>9.677</td>
</tr>
<tr>
<td>Control + 70 % ethanolic extract 20 µg</td>
<td>0.263±0.003***</td>
<td>16.129</td>
</tr>
<tr>
<td>Control + 70 % ethanolic extract 25 µg</td>
<td>0.253±0.003***</td>
<td>19.354</td>
</tr>
<tr>
<td>Control + 70 % ethanolic extract 50 µg</td>
<td>0.203±0.003***</td>
<td>35.483</td>
</tr>
<tr>
<td>Control + 70 % ethanolic extract 100 µg</td>
<td>0.116±0.003**</td>
<td>64.516</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M., n=3
Significance ***P<0.001, **P<0.01, *P<0.05, compared to standard.
Std: Sodium metabisulphate

3. RESULTS AND DISCUSSION
Hydroxyl radical is extremely reactive, most common, highly damaging oxygen species which is generated in our body. It affects almost all components of cells. It is clear from results of hydroxyl scavenging activity that this extract showed a good scavenging activity of 64.516%. The invitro-antioxidation offered by AGEE may be due to the presence of antioxidant phytoconstituents like flavonoids, phyto sterols and other polyphenolic constituents. Therefore this extract showed a very good antioxidant activity. This finding adds strength to our claim.
4. CONCLUSION
AGEE has a good *in-vitro* antioxidant property which is attributed due to the presence of antioxidant phytoconstituents. Therefore the above findings revels that the use of *Amaranthus gangeticus* leaves in our food, protects our vital organs, from various types of diseases.

5. SCOPE FOR FUTURE STUDY
As it is a medicinal plant, hence isolation of its phytoconstituents is needed to screen various organ protective potentials.

6. ACKNOWLEDGEMENTS
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7. REFERENCES

1. www.google.co.in – Wikipedia, the free encyclopedia.