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RESEARCH ARTICLE

COMPARISON OF INVITRO ANTIOXIDANT ACTIVITIES AMONG TANDULIYA (*Amaranth* sps.)

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ABSTRACT

The family Amarantaceae has got potent antioxidant property and in the present study antioxidant properties of the ethyl acetate extracts of *Amarantus viridis* L. and *Amarantus spinosus* L. were evaluated by different in-vitro experiments including DPPH radical assay, Total antioxidant assay and Reducing activity assay for ascorbic acid equivalents, Total Phenolic content and Total flavonoid content. The present study revealed that *Amarantusspinosus* L. extract exhibited the highest DPPH radical scavenging activity (IC50 value of 295.2 µg/ml), Total antioxidant activity (0.25±0.02), Total flavonoid contents (1.50±0.340mg/gm) and *Amarantus viridis* L. extract showed highest Reducing power activity (1.25±0.11),Total phenol content (8.68±0.403mg/gm). The results obtained in the present study indicate that the leaves of Tanduliya showed potential antioxidant and free radical scavenging activity.

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INTRODUCTION

Plants are considered as a treasure of different medicinal properties, which is being used for different ailments from recent past. Tanduliya as the name indicate is an Ayurvedic name of *Amarantus viridis* L. and *Amarantus spinosus* L. commonly called as ceruchira and mullenchira in Indian medicinal plants list. The selected plants are used has a vegetable drug in many ayurvedic preparation and has got antioxidant potential to scavenge the free radicals present in the human body. Synthetic antioxidant like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propylgallate (PG) and tertiary butyl-hydroquinone (TBHQ) are known to ameliorate oxidative damages but they have been restricted due to their carcinogenic and harmful effect on the lungs and liver (Gokhan Zengin *et al*., 2011). Therefore, investigations of antioxidants are focused on naturally occurring substances, especially plant phytochemicals. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds such as gallates which has strong antioxidant activity, while others such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals (Rumit Shah *et al*., 2010). Thus comparative studies on the invitro antioxidant potential among the vegetable drugs *Amarantus viridis* L. and *Amarantus spinosus* L. were assessed in different plants using DPPH radical assay, Total antioxidant assay and Reducing activity assay for ascorbic acid equivalents, Total Phenolic content and Total flavonoid content.

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MATERIALS AND METHODS

Plant material

The plant materials of *Amarantus viridis* L. And *Amarantus spinosus* L. were collected, identified and authenticated. The fresh leaves with petiole were air dried at room temperature for 3 weeks and then grounded into powdered form and then stored in a cool dark place.

Estimation of Phenol

The Folin-Ciocalteu assay using catechin as standard was used for the qualitative test of total phenol from methanolic extracts of the selected plants of *Amarantus* L. The sample extract were mixed with 5 ml Folin-Ciocalteu reagent. After 5 minutes add 4 ml of 7.5% Na_2CO_3 was added to each test tube. After 2 hrs of incubation in dark, the absorbance of the reaction mixture was measured at 740 nm using UV-Visible Spectrophotometer.

The result was expressed as Phloroglucinol equivalent (PGA) in mg/gm of dried sample (Omoruyi *et al*., 2011).

Estimation of Flavonoids

Aluminium chloride colorimetric method was used for the determination of flavonoids. Each plant extracts (0.5ml) in methanol were separately mixed with 0.3 ml of 5%$NaNO_2$. After 10 minutes0.3 ml of 10% aluminum chloride was added and at the 6th minute add 2ml 1M NaOH and 2.8 ml of distilled water. The solution is mixed and the absorbance of the reaction mixture was measured at 510nm with Thermoevolution UV-Visible double beam spectrophotometer (Chang *et al*., 2002).

DPPH Radical Scavenging Activity

10 ml of the different concentrations of leaf extract/standard was centrifuged at 3000 rpm using a centrifuge for 10 minutes and supernatant collected. The supernatant of the extract (1 ml) was added to 3 ml of methanolic solution of DPPH (20 mg/l) in a test tube. The reaction mixture was kept at dark for 15 minutes .The absorbance of the residual DPPH solution was determined at 517 nm in a UV-Visible Spectrophotometer. The experiment was performed in triplicate. Ascorbic acid was used as positive control (Joseph Francis Morrison and Sylvester KwadwoTwumasi, 2010). The inhibition was calculated in following formula,

$$I\ (\%) = 100 \times (A_0 - A_1)/A_0$$

Where A0 is the absorbance of the control, A1 is the absorbance of the extract/standard, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for % 50 inhibition was determined and expressed as IC_{50} value. The lower the IC_{50} value indicates high antioxidant capacity.

Total antioxidant activity

The total antioxidant activity was eluted by using the method described (Prieto *et al*., 1999). Plant extracts were dissolved in methanol to obtain a concentration of 500 µg/ml. 3 ml of extract was placed in a test tube, 0.3 ml of reagent solution (0.6 M Sulphuric Acid, 28 mM Sodium Phosphate, 4 mM Ammonium molybdate) was then added and the resulting mixture was incubated at 950C for 90 minutes.

After the mixture was cooled to room temperature, the absorbance of the each solution was measured by using UV-Visible spectrophotometer at 695 nm against blank. The experiment was performed in triplicate. A calibration curve was constructed, using ascorbic acid (100-1000 µg/ml) as standard and total antioxidant activity of extract was represented in absorbance value.

Reducing Power Activity

2.5 ml of different concentrations of extract/standard was mixed with phosphate buffer (2.5 ml, 0.2 M, PH 6.6) and potassium ferricyanide (2.5 ml, 1%). This was incubated at 50°C for 20 min. After the incubation, 2.5 ml of 10% trichloroacetic acid was added. 2.5 ml of the reaction mixture was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%). The solution absorbance was measured at 700 nm. The experiment was performed in triplicate. Ascorbic acid was used as positive control. Increase in absorbance of the reaction mixture indicated the increased reducing power of the samples (Pandey Manisha *et al*., 2009).

Statistical analysis

Results were given as mean± standard deviation of 3 replicates. The results are expressed as mean values and standard deviation (SD).

RESULTS AND DISCUSSION

The present study was carried out on the plant samples revealed the presence of medicinally active constituents like phenol and flavonoid and also the antioxidant capacity of the plants. The highest total phenol content was recorded in *A. viridis* (8.6±0.403) whereas the total flavonoid content was shown by *A. spinosus* (1.50±0.340) as represented in table 1. Table 2 showed that the DPPH activity of the plants determined by IC_{50} values of ethyl acetate extract of the leafy vegetables, *A. spinosus* shows highest activity since IC_{50} value was 295.2µg/ml than *A. viridis*, which was similar to the reports (Negro *et al*., 2003; Ramadeep and Geoffrey, 2005) shown, that there was a correlation between the antioxidant activity and amount of total phenolics or total flavonoids. Figure 1 and 2 shows the comparison of antioxidant activity especially total antioxidant activity (TAC) and Reducing power assay, result showed that *A. viridis* has good reducing power (1.25±0.11nm) while *A. spinosus* has better total antioxidant capacity (0.25±0.02nm).

Table 1. Total phenol and flavonoid content of the plants

Plant name	Total phenol content (%Mean±SD)	Total flavonoid content (% Mean±SD)
Amarantusspinosus L.	6.04±0.628	1.50±0.340
Amarantusviridis L.	8.6±0.403	1.45±0.400

Table 2. Invitro antioxidant activity of the plants

Plant name	DPPH(IC_{50} µg/ml)	Reducing power(nm)	TAC(nm)
Amarantusspinosus L.	295.2	1.11±0.07	0.25±0.02
Amarantusviridis L.	344.4	1.25±0.11	0.24±0.03
Ascorbic acid	393.3	1.33±0.04	1.13±0.07

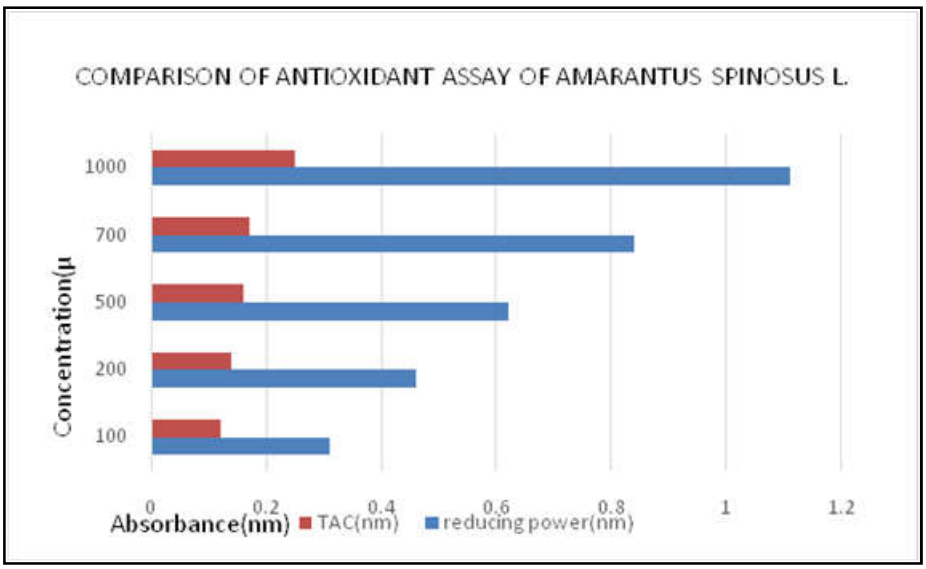

Fig. 1. Comparison of antioxidant assay in *Amarantus spinosus* L.

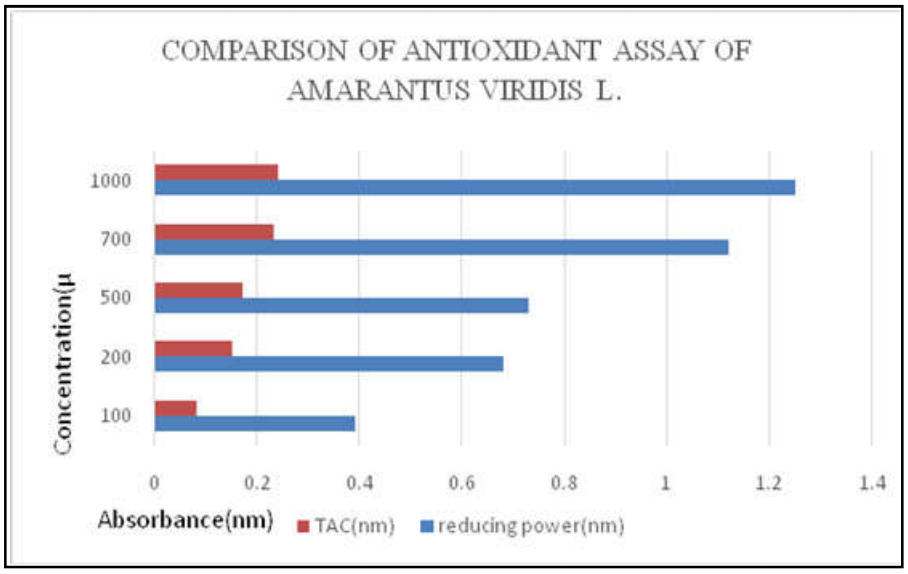

Fig. 2. Comparison of antioxidant assays in *Amarantus viridis* L.

A strong relationship between the total phenolic content and reducing activity in fruits and vegetables has been reported (Yildrim *et al*., 2001). Therefore, the reducing power of the extract may be attributed to its phenolic content.

Conclusion

On the basis of results obtained from different antioxidant capacity assays and estimation of secondary metabolites, the selected plants possess good activity. Furthermore, the determination of total phenolics and flavonoid content along with antioxidant assay of ethyl acetate extract of Tanduliya leaves showed that this plant can be one of the potential sources of safer natural antioxidants. Thus the edible plant species from underutilized plant family had a rich amount of valuable ingredients that are beneficial for health.

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