Clemente and Desai / Journal of Pharmacy Research 2012,5(3),1596-1599

Research Article
ISSN: 0974-6943

Available online through
http://jprsolutions.info

Evaluation of the total phenolic content and primary antioxidant activity of various extracts of *Amaranthus tricolor* Linn.

Aruna Colaco e Clemente and Prakash Vasantrao Desai*

Physiology Laboratory, Department of Zoology, Goa University, Taleigao Plateau, Panaji, Goa 403206, India.

Received on:10-12-2011; Revised on: 15-01-2012; Accepted on:12-02-2012

ABSTRACT

The total phenolic contents and antioxidant potentials of aqueous dried leaf (AD), methanolic (MN & MS) and petroleum ether (PE) extracts of *Amaranthus tricolor* were evaluated. The free radical scavenging activity, ferric reducing ability and total antioxidant capacity of the extracts were tested using three different in vitro antioxidant systems (2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP) and Phosphomolybdenum assays). The AD extracts exhibited the highest total phenolic contents, while PE extracts exhibited the least. AD and PE extracts exhibited significant antioxidant activities but MN and MS extracts showed lowest activities. However the total antioxidant capacity as depicted by phosphomolybdenum assay was higher in MN than in PE extracts. A strong correlation between the DPPH and FRAP values was observed (R^2=0.8390). The antioxidant assays also showed significant correlations with the total phenolic contents.

Key words: *Amaranthus tricolor*, Antioxidant, Phenolic, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP)

INTRODUCTION

Plants have been investigated for their medicinal properties throughout the world, mainly due to their potent pharmacological activities, low toxicity and economic viability.[1] It is well documented that indigenous antioxidants avert the harmful effects of oxidative stress, and therefore increasing the interest in the protective biochemical functions of such natural antioxidants of plant origin.[1, 4]

In the last decade the hunt for naturally occurring antioxidants has grown tremendously since free radicals are known to quench various ailments that affect human health. Consumption of herbal antioxidants improves health. This necessitates the search for natural plant products which could effectively intervene in the onset and morbidity of the disorders and diseases.[5] It is well documented that expression of many disorders or ailments/diseases is associated with the generation of reactive oxygen species or free radicals.[6, 7] Reactive oxygen species (ROS) are often produced as by-products of biological reactions or as a result of intake of exogenous elements which foster oxidative damage,[2] leading to a wide range of biological malfunctions prompting DNA damage, carcinoma, cardiovascular, metabolic and neuro-degenerative disorders, as well as acceleration of senescence.[3, 8] A potent broad spectrum ROS scavenger may serve as a possible preventive intervention for mitigating free radical mediated cellular damages and diseases.[6]

With increased awareness of ROS mediated diseases; worldwide people are turning to alternative herbal medicines rich in antioxidants, with almost no side effects in comparison to allopathic therapies. Phenolic compounds with high antioxidant properties are reported to heal free radical mediated diseases including diabetes mellitus.[9, 10] Amaranthus is one of such plants known traditionally for its healing effects, nutritional and medicinal values,[11, 12, 13 14] while *Amaranthus tricolor* is known for its purple pigments, amaranthine and isoamaranthine,[15] as well as for its potent antidiabetic; antihyperlipidemic,[4] antiviral,[16] antitumor,[17] and hepatoprotective activities[18].

The present study therefore seeks to investigate the antioxidant properties of various solvent extracts of *A. tricolor* and their correlations, if any with the phenolic contents.

***Corresponding author.**
Prakash Vasantrao Desai
Physiology Laboratory,
Department of Zoology, Goa University,
Taleigao Plateau, Panaji,
Goa 403206, India.

MATERIALS & METHODS

Fresh *Amaranthus tricolor* plants were collected from a local source in Fatorda, Goa, India. The plant was identified by Department of Botany, Goa University and a voucher specimen (GUBH-PVAC-0515) was deposited for future reference. The leaves were plucked, rinsed in cold distilled water and dried at 50°C overnight in an oven to a constant weight. The dried leaves were ground and sieved.

Preparation of extracts:

The aqueous extract from dried leaves (AD), was prepared by boiling ten grams of dry leaf powder with 100 ml of water for one hour and the resulting decoction was filtered. Methanolic extracts (MN) was prepared by mixing and shaking of ten grams of dry leaf powder at 27-28°C with 100 ml of methanol for 24 hrs and resulting extract was filtered. For the methanolic soxhlet extract (MS), ten grams of dried leaf powder was soxhelated with 100 ml of methanol at 70°C for one hour and the resulting extract was filtered. Petroleum ether extract (PE) was obtained by mixing ten grams of dry leaf powder with 100 ml of petroleum ether at 70°C for four hours and the resulting extract was filtered. All filtered extracts were evaporated separately at 40°C and the dried extracts were reconstituted (one mg/ml) with the respective solvents before using for the assays.

Total Phenolic Content:

The total phenolic content of the aqueous (AD), methanolic (MN & MS) and petroleum ether (PE) extracts were assayed using the Folin-Ciocalteu method[19]. Both, samples and tannic acid standards were prepared in triplicate. Stock standard solution of tannic acid was prepared by dissolving one microgram of tannic acid in one millilitre of extracting solvent and diluting to 50 ml of distilled water. Working standards were prepared as required by

diluting the stock with distilled water. Plant extracts (100 µl) were incubated at RT along with 0.75 ml of Folin-Ciocalteu reagent for five minutes. Subsequently an equal quantity of six percent (w/v) Na_2CO_3 was added and mixed gently. After 90 min incubation period (RT), absorbance was read at 725 nm (Nanodrop UV-Vis spectrophotometer, USA). The standard calibration curve of tannic acid (0.002–0.2 mg/ml) was plotted and the results expressed as tannic acid equivalents (TAE/gm of dry plant material).

Analysis of antioxidant activity

The antioxidant activities of the aqueous, methanolic and petroleum ether extracts were measured by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), [20] Ferric Reducing Antioxidant Power (FRAP) [21] and Total Antioxidant Capacity (TAC) [22] assays respectively.

DPPH Assay:

Samples were prepared for measuring antioxidant activity by dissolving evaporated extracts (1mg/ml) in methanol.

Qualitative Assay:

Plant extracts (50µL) mixed with 0.1% methanolic DPPH (100 µL) were incubated for 30 minutes at room temperature in dark. The endpoint was reached when the samples turned from purple to yellow to a pale pink indicating a weak to strong antioxidant activity.

Quantitative assay:

Suspension of plant extract (100 µl) and 2.7 ml of methanol + 200 µl methanolic DPPH (0.1 %) was prepared as sample, while a mixture of 200µl methanolic extract (0.1%) and methanol (2.8ml) represented control. The suspension thus prepared was incubated for 30 minutes at room temperature in dark. The absorption maxima of the suspensions were measured spectrophotometerically (UV-2450 Shimadzu, Japan) at 517 nm. The antioxidant activity of the samples was compared with known standard BHT (butylated hydroxytoluene = 0.16%).

The DPPH radical scavenging activity was calculated according to Eq1.

$$\%\ Inhibition = \frac{100(C - S)}{C} \quad \text{----------(1)}$$

Where C and S are the absorbance of control and test samples respectively, at 517 nm.

FRAP activity was calculated according to Eq 2. Where C and S are the absorbance of control and test samples respectively, at 517 nm.

FRAP Assay:

A mixture of freshly prepared working FRAP reagent (1.5ml) and plant extract (50µl) was vortexed. The absorbance (at 593nm) was measured at 0 time. Thereafter, the samples were placed in a water bath (37ºC) and absorption was measured after 4 min. The standard ($FeSO_4$) was processed in the same way.

FRAP activity was calculated according to Eq 2.

$$FRAP\ value = \frac{Sa \times FS}{St} \quad \text{----------(2)}$$

Where Sa and St are the changes in absorbance of the sample and standard respectively, between the zero and the fourth min, and FS is the FRAP value of the standard.

Phosphomolybdenum Assay (Total Antioxidant Capacity):

The total antioxidant capacity was evaluated as described by Prieto et al.[22] Plant extracts (0.1 ml) with one ml of phosphomolybdenum reagent (0.6 M H_2SO_4, 28mM Na_2PO_4 and 4mM $[NH_4]_6Mo_7O_{24}.4H_2O$ was incubated at 95°C for 90 min. Absorbance (695 nm) of samples was measured at room temperature. Separate blanks were made by adding 0.1 ml of extracting solvent to one ml of phosphomolybdenum reagent. Blanks were incubated as described for test solutions. Standard tannic acid (one mg/ml) was used as an internal standard. Antioxidant capacity is expressed as the number of equivalents of ascorbic acid (AAE) produced in the reaction.

Data Analysis:

The results obtained were expressed as mean of triplicate ± SD. Correlation analysis of the total phenolic content versus the phosphomolybdenum assay and the antioxidant activities were carried out using the correlation function in Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

The aqueous (AD) extracts exhibited higher scavenging activity than petroleum ether (PE) extracts (Table 2). Both methanolic (MN and MS) extracts had very low DPPH scavenging activity (Table 1 and 2). The activities of AD and PE were comparable with commercial antioxidant butylated hydroxytoluene (BHT). The total antioxidant activity of the different extracts is presented in Table 3 along with the total phenolic content of each extract. The total antioxidant capacity was found to be highest in AD, followed by

Table 1: Qualitative 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay

DPPH Antioxidant Colour Change	
Aqueous (AD)	Dark to light yellow
Petroleum Ether(PE)	Dark to light yellow
Methanol Normal (MN)	No colour change
Methanol Soxhlet (MS)	No colour change
Butylated hydroxytoluene (BHT)	Colourless

The values calculated as average of triplicate (n=3) ± SD.

Table 2: Antioxidant activities by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays.

Extract	DPPH (% inhibition)	FRAP (mM)
Aqueous (AD)	64.65 ± 0.39	14.26
Petroleum Ether(PE)	64.35 ± 0.84	1.46
Methanol Normal (MN)	2.68 ± 0.23	-6.1333
Methanol Soxhlet (MS)	5.46 ± 0.27	-5.8666
Butylated hydroxytoluene (BHT)	78.11 ± 0.04	-
$FeSO_4$	-	2

The values calculated as average of triplicate (n=3) ± SD.

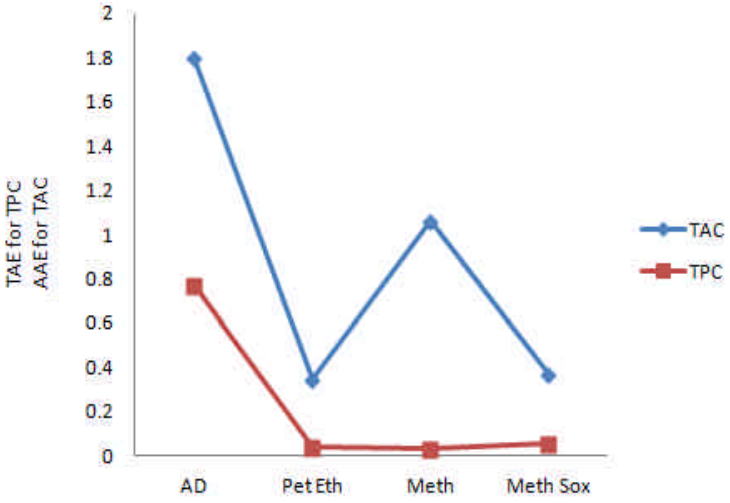
Table 3: Correlation coefficients (R2) between antioxidant activities and Total Phenolic Content

	FRAP	TAC	TPC
DPPH	0.839069	0.233614	0.574214
FRAP	-	0.665343	0.926856
TAC	-	-	0.825426
TPC	-	-	-

DPPH *2,2-Diphenyl-1-Picrylhydrazyl*
FRAP *Ferric Reducing Antioxidant Power*
TAC *Total Antioxidant Capacity*
TPC *Total Phenolic Content*

MN and comparatively lower in MS and PE. The potent DPPH scavenging activity indicates the presence of polyphenols [23, 24] in Amaranthus suggesting its utility in mitigating free radicals effects.

As shown in Fig. 1, AD is seen to have the highest total phenolic content, as compared to the rest of the extracts, which contained significantly lower total phenolic content. When compared with other plants containing high phenolic content, *Amaranthus tricolor* contains a reasonable quantity of phenolic contents, indicating it as a potential source of naturally occurring polyphenols.



The values calculated as average of triplicate (n=3).

Fig.1: Total Phenolic Content and Total Antioxidant Capacity of various extracts of *Amaranthus tricolor*

The total antioxidant capacity and total phenolic contents of the AD, PE and MS extracts correlate with each other (Table 3). But the MN extract does not show any correlation at all with total phenolic contents. The MN extract shows moderate antioxidant activity as compared to that of aqueous extract (AD) but shows very low total phenolic contents. However it is also possible that other phytoconstituents besides the polyphenols may have antioxidant activity.

The present work corroborates the general belief that antioxidant activities go hand in hand with total phenolic contents, but there are reports that there need not be a perfect correlation between antioxidant activity and total phenolic contents of plants since terpenes, sterols when present show antioxidant activity.[25][26] *A. tricolor* is reported to have lipids, polyphenols, tannins and phytosterols as well. [4]

Normally food is enriched by synthetic antioxidants, which unfortunately promote hepatotoxicity and carcinogenicity besides their normal antioxidant activity. [27] Therefore there is an upsurge in demand of natural polyphenols as they retard oxidative degradation of lipids and improve quality and nutritional value of food. [28, 29] Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, thus making it necessary to determine the total amount in the plant material. [5] The significant antioxidant activity exhibited by most of the extracts of *A. tricolor* validates the traditional claim of medicinal value of this plant.

The present study shows that, the aqueous and petroleum ether extracts of *Amaranthus tricolor* exhibit high free radical scavenging activity, as it contains compounds which act as natural scavengers, indicating its possible potential in arresting cellular damage. The findings indicate promising antioxidant capacity of *A. tricolor* and needs to be further investigated to ascertain its full potential in both modern and traditional system of medicines.

ACKNOWLEDGEMENT

This publication is part of ongoing PhD work supported by the University Grants Commission, New Delhi, India, under the UGC-RFSMS scheme and UGC-SAP grants.

REFERENCES

1. Prajapati D, Patel N, Mruthunjaya K, Savadi R. Antioxidant Activity of *Actinodaphne Hookeri* Meissn Leaves. J Sci Res, 1(3), 2009, 606-614.
2. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol, 186, 1990, 1-85.
3. Panayiotidis M. Reactive oxygen species (ROS) in multistage carcinogenesis. Cancer Lett, 266, 2008, 3-5.
4. Clemente CA, Desai PV. Evaluation of the haematological, hypoglycemic, hypolipidemic and antioxidant effects of *Amaranthus tricolor* leaf extract in rat. Trop J Pharm Res, 10(5), 2011, 595-602.
5. Gursoy N, Tepe B. Determination of the antimicrobial and antioxidative properties and total phenolics of two "Endemic" Lamiaceae and *Teucrium chamaedrys* C. Koch. Plant Foods Hum Nutr, 64, 2009, 135-140.
6. Bagchi K, and Puri S. Free radicals and antioxidants in health and disease. East Mediterr Health J, 4(2), 1998, 350-360.
7. Huang W, Cai Y, Zhang Y. Natural Phenolic Compounds From Medicinal Herbs and Dietary Plants: Potential Use for Cancer Prevention. Nutr Cancer, 62(1), 2009, 1-20.
8. Valko M, Dieter L, Jan M, Mark TDC, Mazurn M, Telser J. Free radicals and antioxidants in normal physiological functions and human diseases. Int J Biochem Cell Biol, 39(1), 2007, 44-84.
9. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci, 74, 2004, 2157-2184.
10. Palanisamy UDM. Antioxidant plant extracts as possible anti-hyperglycemic agents. Res J Biotech, 6(3), 2011, 3-4.
11. Pond WG, Lehman JW. Nutritive value of a vegetable amaranth cultivar for growing lambs. J Anim Sci, 67, 1989, 3036-3039.
12. Heever VDE, Venter LS, Peter KV(Ed). Underutilized and underexploited horticultural crops – Amaranth. 4, 2008, 61-92.
13. Odhav B, Kandasamy T, Khumalo N, Baijnath H. Screening of African traditional vegetables for their alpha-amylase inhibitory effect. J Med Plants Res, 4(14), 2010, 1502-1507.
14. Zeashan H, Amresh G, Singh S, Rao CV. Hepatoprotective activity of *Amaranthus spinosus* in experimental animals. Food Chem Toxicol, 46(11), 2008, 3417–3421.
15. Jerz G, Arrey TN, Wray V, Du Q, Winterhalter P. Structural characterization of 132- hydroxy- (132- S) phaeophytin-a from leaves and stems of *Amaranthus tricolor* isolated by high-speed countercurrent chromatography. Innovat Food Sci Emerg Tech, 8(3), 2007, 413-418.
16. Roy S, Sadhana P, Begum M, Kumar S, Lodha ML, Kapoor HC. Purification, characterization and cloning of antiviral/ribosome inactivating protein from *Amaranthus tricolor* leaves. Phytochemistry, 67(17), 2006, 1865-1867.
17. Jayaprakasam B, Zhang Y, Nair MG. Tumor cell proliferation and cyclooxygenase enzyme inhibitory compounds in *Amaranthus tricolor*. J Agric Food Chem, 52(23), 2004, 6939-6943.
18. Al-Dosari MS. The Effectiveness of Ethanolic Extract of *Amaranthus tricolor* L.: A Natural Hepatoprotective Agent. Am J Chin Med, 38(6), 2010, 1051-1064.
19. Amin I, Zamaliah MM, Chin WF. Total antioxidant activity and phenolic content in selected vegetables. Food Chem, 87(4), 2004, 581-586.

20. Benzie IFF, Strain JJ. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem, 239, 1996, 70-76.
21. Wang K, Pan Y, Wang H, Zhag Y, Lei Q, Zhu Z, Li H, Liang M. Antioxidant activities of *Liquidambar formosa* Hance leaf extracts. Med Chem Res, 19(2), 2009, 166-176.
22. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to determination of vitamin E. Analytical Biochem, 269, 1999, 337–341.
23. Gupta S, Prakash J. Studies on Indian green leafy vegetables for their antioxidant activity. Plant Foods Hum Nutr, 64, 2009, 39-45.
24. Kang WY, Li FC, Liu XY. Antioxidant phenolic compounds and **flavonoids of** *Mitragyna rotundifolia* (Roxb.) kuntze in vitro. Med Chem Res, 19, 2010, 1222-1232.
25. Sengul M, Vildiz H, Gungor N, Cetin B, Eser Z, Ercisli S. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak J Pharm Sci, 22(1), 2009, 102-106.
26. Sikder MA, Rahman MA, Islam MR, Kaisar MA, Rahman MS, Rashid MA. Invitro antioxidant, reducing power, free radical scavenging and membrane stabilizing activities of *Spilanthes calva*. Bangladesh Pharm J, 13(1), 2010, 63-67.
27. Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimopoulou AN, Boskou D. Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). Food Chem, 94, 2006, 19-25.
28. Laughton MJ, Halliwel B, Evans PJ, Robin J, Hoult S. Antioxidant and proantioxidant actions of the plant phenolics quercetin, gossypol and myricetin; Effects on lipid peroxidation, hydroxyl radical generation and bleomycin dependant damage to DNA. Biochem Pharmacol, 38(17), 1989, 2859-2865.
29. Bravo L. Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. Nutr Rev, 56(11), 1998, 317-333.

Source of support: UGC, India; Conflict of interest: None Declared