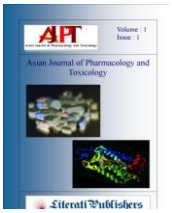
Asian Journal of Pharmacology and Toxicology



RESEARCH ARTICLE

Received on: 01-098-2013
Accepted on: 15-08-2013
Published on: 15-09-2013

Samsul Alam
#260/1, 6th cross, Koramangala 1st block,
Bangalore: 560034
Phone: +919886422393
E mail: dasdev@in.com

QR Code for Mobile users

Conflict of Interest: None Declared !

Evaluation of *in-vitro* antioxidant activity of *Amaranthus tricolor* Linn

Samsul Alam[1*], Krupanidhi K [2], K.R.S Sambasiva Rao [1]
[1]Department of Bio-Technology, Acharya Nagarjuna University, Guntur, A.P, India
[2]Department of Pharmacy, T John College of Pharmacy, Bangalore, India.

ABSTRACT

Objective: To evaluate the antioxidant activity of different extracts of the leaves of *Amaranthus tricolor* Linn.

Methods: The shade dried leaves of *Amaranthus tricolor.*L was extracted with ethanol (95%) and then partitioned by petroleum ether, chloroform and ethyl acetate. The antioxidant activity of various extracts of *Amaranthus tricolor* was evaluated *in vitro* by Free radical scavenging activity (DPPH method) and Nitric oxide scavenging activity assay. Ascorbic acid was used as a reference standard.

Results: The ethyl acetate fraction showed the strongest 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and Nitric oxide scavenging activity among the three fractions.

Conclusion: Our results showed that *Amaranthus tricolor* displayed potent antioxidant properties, supporting the ethno-medical use given to this plant for treatment of diseases.

Keywords: *Amaranthus tricolor* Linn, antioxidant activity, DPPH, nitric oxide

Cite this article as:
Samsul Alam, Krupanidhi K, K.R.S Sambasiva Rao .
Evaluation of *in-vitro* antioxidant activity of *Amaranthus tricolor* Linn.
Asian Journal of Pharmacology and Toxicology 01 (01); 2013; 12-16

1. INTRODUCTION

Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies. About three quarter of the world's population relies on plants and plant extracts for their healthcare [1]. The role of free radicals and reactive oxygen species (ROS) in the pathogenesis of human diseases such as cancer, aging, inflammatory response syndrome, respiratory diseases, liver diseases and atherosclerosis has been widely recognized [2]. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH), as well as non-free radical species (H_2O_2) and the singlet oxygen ($^1O_2$) [3]. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA stand breaking [4]. Therefore, research has focused on the use of antioxidants, with particularly emphasis on naturally derived antioxidants, which may inhibit ROS production and may display protective effects. Plants phenolics, in particular phenolic acid [5-7] tannins [8-9] and flavonoids [10] are known to be potent antioxidant and occur in vegetables, fruits, nuts, seeds, roots, barks and leaves. In addition to their antioxidant properties, these compounds display a vast variety of pharmacological activities [11-12].

A.tricolor (Amaranthaceae) is commonly known as ''Red amaranth" or "Joseph's coat" cultivated throughout South-East Asia and many tropical countries mainly for its edible leaves. *A. tricolor* L. is one of the traditional medicines used in many folk claims and the plant has been extensively used in ayurveda and sidda for treatin g menorrhagia, diarrhea, dysentery, haemorrhagic colit is, bowel hemorrhages, cough and bronchitis. It is also used externally as an emollient poultice or a mouth wash to treat ulcerated conditions of the throat and mouth [13]. Apart from this, the leaves of the plant have been reported to possess wide range of pharmacological activities, like anti-tumor effect [14], anti-ulcer activity [15], hepatoprotective activity [16] and inhibitory effect on cobra venom [17]. Betacyanins, the coloring pigments in *A. tricolor*, have been reported to possess antioxidant activity [18-19].The leaves of *A. tricolor* have been used against external inflammations, as a diuretic, and as a treatment for bladder distress. Phytochemical studies on *A. tricolor* succeeded in the isolation of the antioxidant betacyanins and heteropolysacchrides from the plant [20]. Similarly, Sarkar et al. (2009) revealed that the aqueous extract inhibits the proliferation of a liver cancer cell line (HepG2), breast cancer cell line (MCF-7), and colon cancer cell line (CaCO-2). Furthermore, the whole plant of *A.spinosus,* a synonym of *A. tricolor* has been reported to possess antioxidant activity [21]. However, no study has been conducted to scientifically prove that leaves of *A.tricolor* possess antioxidant activity. Hence, the present study was undertaken to evaluate the *in-vitro* antioxidant effect of the leaves of *A.tricolor*.

2. Materials and methods

a. Collection and Identification of the Plant material

Fresh leaves of *A.tricolor* were collected from Shantipura area of Anekal, India in the month of June. The taxonomical identification of the plant was done by Prof. Balakrishna gowda, GKVK, Bangalore.

b. Chemicals

DPPH was purchased from Sigma Chemicals Co. (St. Louis, MO, USA), while vitamin-E was obtained from Fluka Co. All the solvents used for extraction process and chemicals used for phytochemical analysis were of analytical grade and procured from local firms.

c. Extraction and preliminary phytochemical investigation

The collected leaves of the plant were shade dried and reduced to coarse powder in a mechanical grinder and passed through sieve No. 40. The powdered material obtained was then subjected individually to extraction by cold maceration using rectified spirit (90%) for a total of seven days. The extracts were filtered and concentrated in rotary evaporator under reduced pressure to yield a thick green ethanolic extract. The crude extract thus obtained was partition-fractionated with 1:1 of petroleum ether and ethanol (50%), the mixture was shaken vigorously and kept for about 30 minutes to let the two layers separate. The upper layer consisted of petroleum ether, it was removed and concentrated in a rotary evaporator to obtain petroleum ether fraction (PEAT). The same procedure was repeated with the residue using equivalent volume of chloroform and ethyl acetate to obtain chloroform fraction (CAT) and ethyl acetate fraction (EAAT) respectively. The extracts thus obtained were subjected to phytochemical analysis [22].

d. In-vitro antioxidant activity

2.4.1 Free Radical Scavenging Activity (DPPH Method)

The radical scavenging activity of all the plant extracts/isolated constituents was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) as described by Molyneux [23]. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 1.0 ml of extract solution in methanol at different concentrations (5 µg/ml-500

µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Thirty minutes later, the absorbance was measured at 517 nm and ascorbic acid was used as a reference standard at concentrations ranging from 5-50 µg/ml. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The percent inhibition was calculated from the following equation:

% inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance control] x100

The antioxidant activity of the extracts/isolated constituents was expressed as inhibitory concentration (IC). IC_{50} is defined as the concentration in µg/ml of extracts sufficient to obtain 50% of a maximum scavenging capacity.

2.4.2 Nitric oxide scavenging activity assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions. This can be determined by the use of the Griess Illosvoy reaction [24], 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25^0C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 546 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated according to the following equation:

NO scavenged (%) = ((A0-A1) / A0 × 100)

Where, **A0** = absorbance of the control (blank, without extract), **A1**= absorbance in the presence of the sample/standard.

e. Statistical analysis

Data were presented as mean ± standard deviation (SD) of three determinations. Statistical analyses were performed using a one-way analysis of variance. The IC_{50} values were calculated by linear regression analysis. Results were calculated by employing the statistical software (SPSS 13.0, SPSS Inc., USA).

3. Results

a. Preliminary Phytochemical Investigation

Preliminary phytochemical screening of the leaf extracts revealed the presence of steroids, triterpinoids and saponins in PEAT and CAT. EAT showed the presence of alkaloids, carbohydrates, proteins, saponins, flavonoids, tannins and glycosides.

b. DPPH radical scavenging activity

As shown in **Table-1** *Amaranthus tricolor* Linn. leaf extracts strongly scavenge in dose dependent manner. IC50 value for ethanolic extract, ethyl acetate extract and ascorbic acid was found to be 849, 206 and 103µg/ml respectively

Extract	Quantity in micrograms (µg/mL), Mean ±SEM					IC_{50}
	50	100	200	500	1000	
EAT	3.21±1.28	14.7±1.07	21.8±1.58	39.7±1.98	58.9±1.57	849
PEAT	1.10±0.68	11.7±0.87	15.5±1.27	19.8±0.58	28.6±0.93	1748
CAT	2.14±1.41	4.52±0.78	9.17±1.72	18.4±0.94	37.5±1.08	1333
EAAT	24.6±1.44	38.0±0.81	48.5±0.59	62.1±1.75	73.9±0.48	206
AA	36.08±0.89	48.50±0.69	68.12±0.72	82.79±1.75	95.05±2.07	103

AT: *A.tricolor*. L, **EAT:** Ethanolic extract of AT, **PEAT:** Petroleum ether extract of AT, **CAT:** Chloroform extract of AT**, EAAT:** Ethyl acetate extract of AT, **AA:** Ascorbic acid.

Table–1: Percentage inhibition and IC50 values of DPPH radical in-vitro by ethanolic extract, petroleum ether, chloroform, and ethyl acetate fractions of *Amaranthus tricolor* leaves and ascorbic acid.

c. Nitric oxide scavenging activity

As shown in **Table-2** *Amaranthus tricolor* Linn. leaf extracts strongly scavenge in dose dependent manner. IC50 value for ethanolic extract, ethyl acetate extract and ascorbic acid was found to be 1.71, 1.28 and 0.052mg/ml respectively

Extract	Quantity in milligrams (mg/mL), Mean ±SEM						IC_{50}
	0.05	0.25	0.50	1.00	2.00	3.00	
EAT	4.45± 1.59	11.2± 1.14	24.2 ± 2.16	29.2± 2.27	57.4+2.11	96.2± 3.75	1.71
PEAT	1.08±0.89	2.80±0.69	8.58±0.72	15.2±1.75	26.5±2.07	29.9±2.07	5.01
CAT	1.28 ± 0.21	5.97± 1.00	14.9± 1.13	21.9±2.22	26.8± 1.67	32.3± 1.67	4.64
EAAT	3.87± 0.43	12.8± 1.42	26.8 ± 0.78	38.9 ± 1.16	53.2± 0.42	74.3± 0.42	1.28
AA	48.3 ± 1.01	66.2± 0.21	79.1± 0.48	88.2± 0.75	95.1± 1.57	98.1± 0.14	0.052

AT: *A.tricolor*. L, **EAT:** Ethanolic extract of AT, **PEAT:** Petroleum ether extract of AT, **CAT:** Chloroform extract of AT**, EAAT:** Ethyl acetate extract of AT, **AA:** Ascorbic acid.

Table–2: Percentage inhibition and IC50 values of nitric oxide radical scavenging activity in-vitro by ethanolic extract, petroleum ether, chloroform, and ethyl acetate fractions of *Amaranthus tricolor* leaves and ascorbic acid.

4. DISCUSSION

DPPH is stable nitrogen centered free radical that can accept an electron/hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agent than losing stiochiometrically with the number of electrons consumed which is measured spectrometrically at 517 nm. It has been reported that a series of human illness such as cancer, atherosclerosis, neurodegenerative diseases, cerbrovascular diseases, Alzheimer's diseases and cardiac diseases, can be linked to the damaging action of extremely reactive free radical [25]. Ascorbic acid is a potent free radical scavenger, so when compared to such pure component, IC_{50} of ethanolic and ethylacetate extracts shows that *A.tricolor* is potent free radical scavenger. In very recent year, potent free radical scavengers have attracted a tremendous interest as possible therapeutic against free radical mediated diseases.

Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities [26]. Although nitric oxide and superoxide radicals are involved in host defense, over production of these two radicals contributes to the pathogenesis of some inflammatory diseases [27]. Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules. Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases. Leaf extracts of *A.tricolor* significantly inhibited nitric oxide in a dose dependent manner. These results showed the ability of the leaf extracts of *A.tricolor* to reduce free radicals which may stop the free radical initiation or retard free radical chain reaction in the propagation of the oxidation mechanism.

5. CONCLUSION

The data presented here indicate that the marked antioxidant activity of Leaf extracts of *A.tricolor* seems to be due to presence of flavonoids like flavones, flavanes and flavonols which act in similar fashion as redutones by donating the electrons and reacting with free radicals to convert them into more stable product and terminate free radical chain reaction. In addition, these results form a good basis for selection of the plant for further pharmacological investigation. The present study supports the folkloric usage of this plant.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This research project was supported by management of T John College of Pharmacy, Bangalore, India. The authors are also thankful to Prof. Balakrishna gowda, GKVK, Bangalore for identifying the plant and for his valuable suggestions during the work. The grant No: TJ-979

6. REFERENCES

1. Shivanna Y, Koteshwara AR. *In-vitro* antibacterial effect of selected medicinal plant extracts. *J Nat Prod* 2009; **2**:64–69.
2. Pallavi S, Ambuj BJ, Rama SD, Mohammad P. Reactive oxygen species, oxidative damage, and anti-oxidative defense mechanism in plants under stressful conditions. *J Botany* 2012; **2012**: 1–26.
3. Adedapol AA, Jimoh FO, Afolayan AJ, Masika PJ. Antioxidant properties of the methanol extracts of the leaves and stems of *Celtis Africana*. *Rec Nat Prod* 2009; **3**:23–31.
4. Nan Wu, Kuang Fu, Yu-Jie Fu, Yuan GZ, Fang RC, Yung HC, Xiao LL, Kong Y, Wei L, Cheng BG. Antioxidant activities of extracts

and main components of Pigeonpea [*Cajanus cajan* (L.) Millsp] leaves. *Molecules* 2009; **14**: 1032–1043.
5. Duthie G, Morrice P. Antioxidant capacity of flavonoids in hepatic microsomes is not reflected by antioxidant effects *in-vivo. Oxid Med Cell Longev* 2012; **2012**: 1–12.
6. Hashimoto K, Sakagami H. Induction of apoptosis by epigallocatechin gallate and autophagy inhibitors in a mouse macrophage-like cell line. *Anticancer res* 2008; **28**: 1713–1718.
7. Siahpoush A, Ghasemi N, Ardakani MS, Asghari G. Production of chlorogenic acid in *Varthemia persica* DC (var. persica) callus cultures. *Res Pharm Sci* 2011; **6**:63–69.
8. Shu-Jing W, Lean-Teik N. Antioxidant and free radical scavenging activities of wild bitter melon (*Momordica charantia* Linn. var. abbreviata Ser.) in Taiwan. *LWT* 2008; **41**:323–330.
9. Okuda T. Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochem* 2005; **66**: 2012–2031.
10. Padmanabhan P, Jangle SN. Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations. *Inter J Pharm Sci Drug Res* 2012; **4**: 143-146.
11. Ganesh NS, Susheel KD, Nitin S, Jyotsana S. Anti-inflammatory Activity and Total Flavonoid Content of Aegle marmelos Seeds. *Int J Pharm Sci Drug Res* 2011; **3**: 214-218.
12. Owen RW, Giacosa A, Hull WE, Haubner R, Bartsch H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *Eur J Cancer* 2000; **36**: 1235–4127.
13. Misra RC. Therapeutic uses of some seeds among the tribals of Gandhamardan hill range, Orissa. *Ind. J. Trad. Know.* 2004; **3**: 105–115.
14. Bolleddula J, Zhang Y, Nair MG. Tumor cell proliferation and cyclooxygenase enzyme inhibitory compounds in *Amaranthus tricolor*. *J. Agric. Food Chem.* 2004; **52**: 6939–6943.
15. Zeashan H, Amresh G, Singh S, Rao CV. Antidiarrheal and antiulc er activity of *Amaranthus spinosus* in experimental animals. *Pharmaceutical biol*. 2009; **47**:932–939.
16. Mohammed SA. The effectiveness of ethanolic extract of *Amaranthus tricolor* L.: A natural hepatoprotective agent. *Am. J. Chin. Med*. 2010; **38**: 1051–1064.
17. Daduang S, Sattayasai N, Sattyasai J, Tophram P, Thammathaworn A, Chaveerach A, Konchaiyaphum M. Screening of plants containing Naja najasiamensis cobran venom inhibitory activity using modified ELISA technique. *Anal. Biochem.*2005; **341**: 316–325.
18. Henriette MCA. Betalains: properties, sources, applications, and stability – a review. *Int. J. Food Sci. Tech.* 2009; **44**:2365–2376.
19. Cai Y, Sun M, Corke H. Antioxidant activity of betalains from plants of the Amaranthaceae. *J. Agric. Food Chem.* 2003; **51**: 2288–2294.
20. Cai YZ, Xing J, Sun M, Corke H. Rapid Identification of Betacyanins from *Amaranthus tricolor*, Gomphrena globosa, and Hylocereus polyrhizus by Matrix-Assisted Laser Desorption/Ionization Quadrupole Ion Trap Time-of-Flight Mass Spectrometry (MALDI-QIT-TOF MS). *J. Agric. Food Chem.* 2006; **54**: 6520–6526.
21. Ashok Kumar BS, Lakshman K, Jayaveera KN, Khan S, Manoj B, Narayan swamy VB. Evaluation of the Antioxidant Activity of *Amaranthus spinosus* Linn. by Non-Enzymatic Haemoglycosylation. *Sains. Malaysiana.* 2010; **39**: 413–415.
22. Bhagavan SP, Ambarsing PR. Chemical constituents from petroleum ether extract of leaves of *Butea monosperma* and their antimicrobial, antifungal activity. *Int. J. Pharm Tech Res.*2012; **4**: 321–326.
23. Munguia AL, Romero YH, Chaverri JP, Molina AM, Regla I, Martinez A, Castillo E. Phenylpropanoid glycoside analogues: Enzymatic synthesis, antioxidant activity and theoretical study of their free radical scavenger mechanism. *PLoS one* 2011; **6**:1–9.
24. Akiri SVCR1, Sareddy GR2, Phanithi PB, Attipalli RR. The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. *BMC Compl Altern Med* 2010; **10**:1–9.
25. Nijveldt RJ, Nood EV, Van–Hoom DEC, Klaske VN. Flavonoids: a review of probable mechanism of action and potential applications. *The Amer J Clin nutr* 2001; **74**: 418–425.
26. Chowdhury S, Ghosh AK, Maji S, Bala NN. Antioxidant capacities of a flavonoid compound isolated from *C.Salignus* leaves. *Int.J.Pharm. Bio. Sci.* 2012; **3**: 112–118.
27. Isik F, Tugba TA, Yarat A, Genc Z, Pisiriciler R, Cetinel S, Altintas A, Sener G. Protective effects of Black Cumin (*Nigella sativa*) oil on TNBS-Induced experimental colitis in rats. *Diges. Dise. Sci.* 2011; **56**: 721–730.