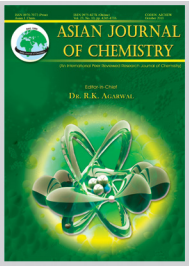
Asian Journal of Chemistry; Vol. 24, No. 1 (2012), 455-460

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Pharmacognostic, Phytochemical, Antimicrobial and Antioxidant Activity Evaluation of *Amaranthus tricolor* Linn. Leaf

THARUN[1], KORLEPARA NAGAVENKATESHWAR RAO[1,*], SAI KRUSHNA PADHY[1], SATHIS KUMAR DINAKARAN[1], DAVID BANJI[1], HARANI AVASARALA[1], SOMSUBHRA GHOSH[1] and MACHARLA SRAVAN PRASAD[2]

[1]Nalanda College of Pharmacy, Nalgonda-508 001, India
[2]GBN Institute of Pharmacy, Ghatkesar, RR District-501 301, India

*Corresponding author: E-mail: raoknv@yahoo.co.in

(*Received*: 4 April 2011; *Accepted*: 27 September 2011) AJC-10458

The study is aimed to investigate the pharmacognostic, phytochemical, antimicrobial screening and antioxidant activity of different extracts of *Amaranthus tricolor* with a view of justifying the use of the plant. The test tube reactions and TLC examination revealed the presence of phenols, tannins and flavonoids in chloroform, ethanolic and aqueous extracts of *A. tricolor*. By present work, it is concluded that the plant *A. tricolor* is endowed with significant antimicrobial and antioxidant activity due to the presence active constituents like polyphenolic conents and others, thus justifying its use in the indigenous system of medicine. The results of organoleptic, microscopical and physical evaluation were satisfactory and can be correlated with earlier reports.

Key Words: *A. tricolor*, Pharmacognostic evaluation, Phytochemical screening, Antimicrobial activity, Wound healing activity.

INTRODUCTION

World is endowed with a rich wealth of medicinal plants. Herbs have always been the principal form of medicine in world. A good number of drugs that are being used today are isolated from plant sources and some from animals or minerals. In the world, 80 % of population reportedly uses herbs for medicinal purposes[1]. Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary metabolites in one or more parts of the plants. *Amaranthus tricolor* Linn is a medicinal plant, which belongs to Amaranthaceae family is the most important species in South Africa follwed by others. It has been used for the treatment of piles, bladder distress, blood disorders, tooth ache and dysentery and even as astringent, diuretic, haemorrhage and hepatoprotective agent[2]. Yellow, green and red dyes can be obtained from whole plant[3]. Amaranth is an erect tropical annual with long-stemmed leaves and small black seeds growing up to 5' tall. The leaves, up to 5" long, are notched or rounded at the tips. The flowers are whitish-green while the seeds are very small, black or red-brown and relatively large with about 1200-2900 seeds/g[4]. *Amaranthus tricolor* is very closely related to the species *Amaranthus spinosus*, *A. hybridus* and *A. dubius*. They are rich in minerals (calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese) and vitamins (vitamin A, vitamin B_6, vitamin C, riboflavin and foliate)[5,6]. Gerold jerz *et al.*[7] isolated two chlorophyll breakdown products 132-hydroxy-(132-S)-phaeophytin-a and chlorophyll b methoxylactone by preparative high-speed countercurrent chromatography' from lipophilic extracts of the aerial parts of *A. tricolor*. The major unsaturated fatty acids in *A. tricolor* are linoleic acid in seeds (49 %) and stems (46 %) and linolenic acid in leaves (42 %), while the major saturated fatty acid in seeds, stems and leaves is palmitic acid at 18-25 % of total fatty acids[8]. Mature leaves of *A. tricolor* and *A. caudatus* contain red violet pigments-the betacyanins amaranthin and isoamaranthin[9]. They are derivatives of betanidin, which is formed from 3,4-dihydroxyphenylalanine[10]. Hence, it was thought worthwhile to carryout the phamacognostic phytochemical antimicrobial screening and antioxidanat activity of different extracts of *A. tricolor* with a view to justifying the use of the plant and proceeded with the present study.

EXPERIMENTAL

Streptomycin injection (Sarabhai Piramal Pharmaceuticals, Vadodara, Gujarat) was purchased from local market. Test organisms, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *Salmonella typhi* (ATCC 19430), *Klebsiella phuemoniae* (ATCC 10031), *Escherichia coli* (ATCC 10536) and *Pseudomonas aeurginosa* (ATCC 9027) were obtained from Department of Microbiology, Nalanda College

of Pharmacy, Nalgonda, India. Nutrient agar, mycological peptone, agar and dextrose were obtained from Himedia labs, Mumbai. All the solvents were procured from E. Merck, Mumbai.

Collection of plant materials: The leaves of *Amaranthus tricolor* were collected from Ankapoor in Nizamabad district of Andhra Pradesh, India. The plant was authenticated by Prof. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati, India. The collected leaves were dried at room temperature and powdered.

Pharmacognostical evaluation: The selected plant *A. tricolor* was subjected to organoleptic (colour, odour and taste of the leaf powder were recorded), microscopic (essential for powdered crude drugs consist of the fragments of cells in the form of recognizable tissues and the study of surface constants like fibres, lignified vessel, epidermal cells, calcium oxalate crystals, starch grains, *etc*.) and physical evaluation (includes some important physical characteristics like ash values, extractive values, moisture content, *etc*.) using standard procedures.

Preparation of extracts: The extracts of leaves of *A. tricolor* were prepared by successive soxhlation with various solvents *viz*. The shade dried leaf powder was packed in thimble kept in the soxhlet apparatus and extraction was allowed to run successively using the solvents, petroleum ether (60-80 ºC), benzene, acetone chloroform and ethanol. Finally, the marc was dried and macerated with chloroform-water for 24 h to obtain the aqueous extract. Each extract was concentrated by evaporating the solvent on the water-bath and the obtained extracts were weighed. The physical characteristics and percentage yield of various extracts were tabulated.

Phytochemical screening: The crude extracts or isolated constituents were subjected to qualitative and quantitative chemical analyses. All the extracts were subjected to preliminary phytochemical screening for the detection of various chemical constituents.

Thin layer chromatography: All extracts were dissolved in their respective solvents and spotted on TLC plates (silica gel GF and silica gel G plates) and developed in appropriate solvent system for identification of various chemical classes[11,12]. Then the plates were dried and the resolution of components of extracts was studied by locating various spots on chromatogram. The positions of the spots identified first by visual observation and then under UV lamp for steroidal glycosides, for carbohydrates, anisaldehyde and sulphuric acid where as for saponins, vanillin and sulphuric acid and for flavonoids, natural products reagents (NP:PEG No:28) and UV lamp. Solvent system used for steroidal glycosides are ethyl acetate(100) : methanol(13.3) : water(10), for carbohydrates are *n*-butanol(7) : ethyl acetate(20) : isopropanol(12) : glacial acetic acid(7) : water(6), for saponins is chloroform(64): methanol(50) : water(10) and for flavonoids benzene(90) : ethyl acetate(120) : glacial acetic acid(5).

Isolation of chemical compounds: This isolation was planned by using Harborne method with little modifications. The powder drug was taken in a beaker then boiled by 2 M HCl for 1 h and filtered followed by cooling and extraction with petroleum ether and ethyl acetate successively. Finally crystalline compound was obtained along with ethyl acetate fraction[13] and petroleum ether which was subjected to different phytochemical tests. Then TLC of isolated compound was developed by using silica gel GF and benzene(9): ethyl acetate (12) : glacial acetic acid (0.5) as solvent system. The positions of the spots identified under UV lamp.

High performance thin layer chromatography: The isolated compound and ethanolic extract were analzed using HPTLC for the presence and quantification of compound. Estimation of total polyphenolic content by UV spectrophotometry. It was done for ethanolic and water extracts of the leaf and the isolated compound. Gallic acid was used as standard. 1 mL of different concentrations (5, 10, 15, 20 and 25 µg/mL) of above was mixed separately with 1 mL of 95 % ethanol, 5 mL of distilled water and 0.5 mL of 50 % Folin-Ciocalteu reagent. Then the mixture was incubated for 1 h in dark and absorbance was measured at 725 nm immediately.

Antimicrobial assay: Petroleum-ether, chloroform, alcoholic and aqueous extracts of *Amaranthus tricolor* were screened for antimicrobial activity by cup plate method. The activity was compared with standard streptomycin and control (DMSO). Various organisms used in the study were gram +ve bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram -ve bacteria (*Klebsiella phuemoniae*, *Escherichia coli*, *Pseudomonas aeurginosa, Salmonella typhi*).

Preparation of solutions: Different concentrations of extracts equivalent to 500, 1000 and 1500 mg/0.1 mL were prepared by using DMSO. 100 mg/ mL concentration of streptomycin was prepared and used as standards to be studied along with test solutions. After incubation period it was studied for their zone of inhibition individually.

Procedure[14]: Nutrient agar was used to study the antibacterial activity of the extracts[15]. 25 mL of media was poured in each petridish of 9 cm diameter so as to obtain 3-4 mm thickness layer of media. After solidification, a sterile borer was used to prepare five cups of 8 mm diameter in the medium of each petri dish. An accurately measured 0.1 mL solution of each concentration of solutions of extracts and standard samples were added to the cups with the help of micropipette. All the plates were kept at room temperature for effective diffusion of extracts and standards. Later they were incubated at 37 ± 1 ºC for 24 h. The zone of inhibition around the cup indicates the antimicrobial activity. The control was run simultaneously to asses the activity of DMSO, which was used as vehicle for extracts. The study was performed in duplicate. The diameter of the zone of inhibition was measured and recorded.

***In vitro* evaluation for antioxidant activity**

DPPH free radical scavenging activity: The DPPH free radical scavenging activity was assessed according to Okada & Okada method[16]. An ethanolic solution of DPPH (0.05 mM, 300 µL) was added to 40 µL of extract solution with different concentrations (0.025-2 mg/mL). The DPPH solution was freshly prepared and kept in the dark at 4 ºC. Ethanol 96 % (2.7 mL) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and the absorbance was measured using a spectrophotometer at 517 nm. Ethanol was used to zero the spectrophotometer. A blank sample containing the same amount of ethanol and DPPH was also

prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation[17]:

Per cent of DPPH inhibition= [(AB-AA)/AB] × 100

where, AA and AB are the absorbance values of the test and of the blank sample, respectively.

Assay of reducing power[18]: 1 mL of plant extract solution (final concentration 20- 100 µg/ mL) was mixed with 2.5 mL phosphate buffer (2 M, pH 6.6) and 2.5 mL potassium ferricyanide [$K_3Fe(CN_6)$] (10 g/L) and then the mixture was incubated at 50 ºC for 20 min. 2.5 mL of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 1500 rpm for 10 min. Finally, 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL $FeCl_3$ (0.1 %) and absorbance measured at 700 nm in UV-visible spectrophotometer (Elico double beam UV-Visible spectophotometer 196, India). Ascorbic acid was used as standard and phosphate buffer as blank solution. The absorbance of the final reaction mixture of two parallel experiments was expresses as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power.

Determination of peroxide radical scavenging activity[19]: Different concentrations of drug ranging from 20-100 µg/ mL in phosphate buffer saline was prepared and incubated with 0.6 mL of 4 mM H_2O_2 solution prepared in PBS for 10 min. The standard ascorbic acid was used as standard and absorbance was measured at 230 nm.

Determination of total antioxidant activity[20]: The tubes containing 0.2 mL of the extracts(100-500 µg/mL), 1.8 mL of distilled water and 2 mL of phosphomolybdium reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95 ºC for 1.5 h. Thus the mixture was cooled to room temperature and the absorbance was measured at 695 nm. The antioxidant capacity was expressed as ascorbic acid equivalent.

RESULTS AND DISCUSSION

In the present study, the leaves of *Amaranthus tricolor* were collected, dried and subjected to size reduction to get uniform coarse powder. The powdered drug was subjected to pharmacognostic evaluation in terms of organoleptic, microscopic and physical characteristics. The organoleptic characters *viz*., colour, odour and taste were observed and the results were greenish red colour, characteristic odour and tasteless. The leaf was found to be simple, 5-12 cm long, lanceolate, obtuse, petiolate and membranous texture. The leaf powder was subjected to microscopic analysis. The analysis revealed the presence of loped epidermal cells with anomocytic stomata, druses or sphaero type of calcium oxalate crystals and narrow and thick walled xylem vessel elements. The results were in agreement with previous reports on leaves. For physical evaluation, the powdered drug was subjected for estimation and the values were found to be 8.6 % of total ash, 1.5 % of acid insoluble ash, 3.6 % of water soluble ash, 6.2 % of water soluble extractive value, 5.8 % of alcohol soluble extractive value and even loss on drying was noticed. These values indicate the difference in chemical composition particularly polar substances present in the plant. The shade dried leaf powder of *A. tricolor* was successively extracted by soxhlation with petroleum ether, benzene, acetone, chloroform, ethanol and water. The percentage yields and nature of extracts were given in Table-1. The powdered drug was subjected to extraction with various solvents such as petroleum ether, benzene, acetone, chloroform, ethanol and water by successive soxhlation and finally the marc was macerated with chloroform water I.P. preliminary chemical analysis of various extracts of *Amaranthus tricolor* was done. From the result, it can be inferred that carbohydrates were present in ethanol and aqueous extracts; glycosides, phenolic compounds, flavonoids and saponins were present in chloroform, ethanol and aqueous extracts and steroids were present in petroleum ether, benzene, acetone and chloroform extracts. Steroidal glycosides, carbohydrates, saponins and flavonoids were analzed in all extracts by TLC. In TLC, the extracts were chromatographed to detect their presence with their respective solvents. R_f values were calculated for all the spots. The results of this analysis were shown in Table-2. From Table-2, it can be inferred that carbohydrates, saponins and flavonoids were not detected in petroleum ether and benzene extracts. Acetone, chloroform and ethanol extract were found to contain saponins and flavonoids based on spot. Carbohydrates were present in acetone and ethanol extract. Steroidal glycosides were found in petroleum ether and benzene extracts. The difference in R_f value may be resulting from the purity of the solvent, nature of substance to be resolved, composition of solvent system, presence of impurities, adsorbent used, polarity of the solvent, substance, absorbent, *etc*.

TABLE-1
PER CENTAGE YIELD AND NATURE OF THE EXTRACT

Extracts	Yield (w/w) (%)	Nature	Colour
Petroleum ether	11.26	Solid	Yellowish green
Benzene	3.3	Semi solid	Green
Acetone	1.33	Solid	Light yellow
Chloroform	2.4	Semi solid	Green
Ethanol	8.66	Solid	Green
Water	9.8	Solid	Brown

TABLE-2
RESULTS OF THIN LAYER CHROMATOGRAPHY ANALYSIS

Compound	R_f value					
	PEE	BE	AE	CE	EE	WE
Steroidal glycosides	0.05	0.04	-	-	-	-
Carbohydrate	-	-		-	0.24 0.56 0.71 0.82	0.86 0.78
Saponins				0.56 0.94	0.97	0.83
Flavonoids				0.26 0.48 0.88	0.45 0.68 0.88	0.26 0.48 0.93

PEE–Petroleum ether extract, AE- Acetone extract, EE–Ethanol extract, BE–benzene extract, WE–Aqueous extract, CE–Chloroform extract, STD–standard compound, Ext-Extract.

Isolation of chemical compound was carried out as mentioned earlier. The petroleum ether and ethyl acetate fraction

and isolated crystalline compound were subjected to different phytochemical tests. The results of this analysis can be expressed that carbohydrates, tannins and flavonoids were detected in ethyl acetate fraction. Steroids were present in petroleum ether fraction. Steroidal glycosides were found in petroleum ether. Isolated compound had shown a positive result to phenolic compounds only. TLC of isolated compound was developed with ethanolic extract of *A. tricolor*. The isolated compound showed a single spot with R_f value of 0.32, which was correlated with the R_f value of a spot occured in ethanolic extract. The HPTLC profile of ethanolic extract gave information regarding 5 spots, R_f values and % area under curve of the different spot in ethanolic extracts were determined 0.13, 0.32, 0.44, 0.66 and 0.73 and 31.74, 20.13, 14.89, 10.32 and 22.93 respectively. The R_f value of an isolated crystalline compound was 0.34 (% area under curve is 38.25) and this value was correlated with one of the R_f value of ethanolic extract(0.32). By the TLC and HPTLC, we may assume that the ethanolic extract having isolated compound along with other compounds. The results obtained in analysis of isolated compounds indicated that ethanolic extracts had shown the presence of phenolic compounds. The HPTLC chromatograms of ethanolic extract and isolated compound were shown in Figs. 1-3, dimensional photographs of isolated compound and ethanolic extract of *A. tricolor* were shown in Fig. 3.

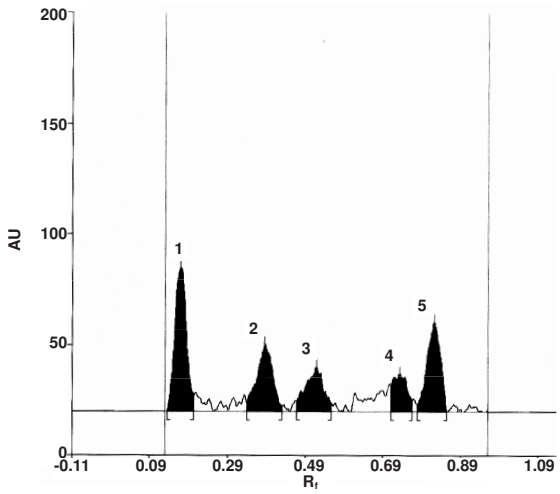


Fig. 1. HPTLC chromatograms of ethanolic extracts

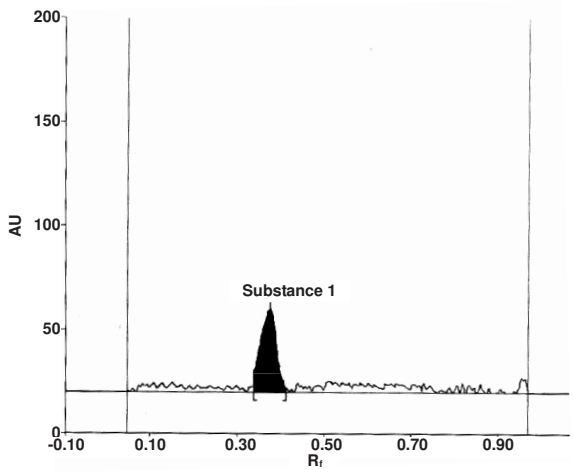


Fig. 2. HPTLC chromatograms of isolated compound

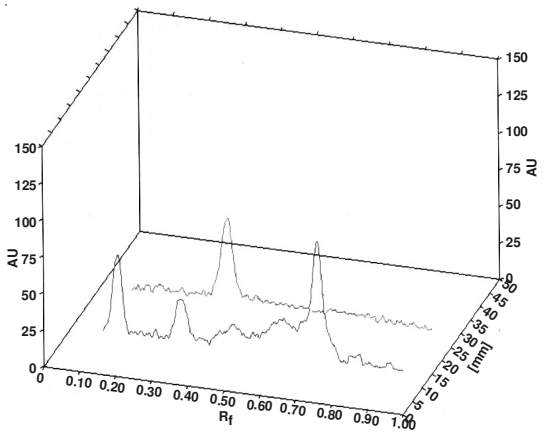


Fig. 3. Three dimensional photograph of isolated compound and ethanolic extract of *A. tricolor*

The total polyphenolic content of ethanolic and water extracts and isolated compound was estimated by UV spectrophotometric method. The results were present in Table-3. From the result it can be known that the water extract showed significant amount of phenolic compound presence to the standard, ethanolic and water extract.

TABLE-3
ESTIMATION OF TOTAL PHENOLIC COMPOUND

Concentration	Absorbance at 725 nm			
	Ethanolic extract	Water extract	Isolated compound	Gallic acid (std)
10	0.004 ± 0.001	0.006 ± 0.001	0.005 ± 0.007	0.14 ± 0.014
20	0.006 ± 0.003	0.076 ± 0.007	0.063 ± 0.002	0.28 ± 0.002
30	0.21 ± 0.002	0.29 ± 0.001	0.27 ± 0.015	0.37 ± 0.006
40	0.26 ± 0.002	0.46 ± 0.001	0.38 ± 0.02	0.54 ± 0.002
50	0.51 ± 0.002	0.69 ± 0.003	0.62 ± 0.15	0.87 ± 0.003

n = 5, mean ± SE

The antimicrobial activity of petroleum ether, chloroform, ethanol and water extracts of leaves of *A. tricolor* against various strains of bacterial microorganisms was evaluated by cup plate method and diameter of zone of inhibition was measured in mm. In the present study antimicrobial activity of single concentrations of streptomycin was also performed. The data obtained for the antimicrobial potential of the standard streptomycin was 6.0 ± 1.9, 6.2 ± 1.2, 6.0 ± 1.9, 6.0 ± 0.2, 9.0 ± 0.1 and 6.0 ± 1.5 for *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella phuemoniae*, *Escherichia coli*, *Pseudomonas aeurginosa* and *Salmonella typhi* respectively. (Mean ± standard deviation, n = 3). In this study, none of the extracts show a zone of inhibition against mentioned microorganism, which was a controversy to the report of Behari *et al.*[21] states that petroleum ether extract of *A. tricolor* showed significant antimicrobial activity due to the presence of steroids.

Petroleum-ether, chloroform, alcoholic and aqueous extracts of *Amaranthus tricolor* leaves and isolated compound were screened for antioxidant activity and results were compared with vitamin C. DPPH radical scavenging activity, reducing power, hydrogen peroxide scavenging activity and total antioxidant activity of leaves extracts of *A. tricolor* and vitamin C were presented in Figs. 4-7, respectively. Generally flavonoids and tannins are phenolic compounds and plant

phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers[22]. The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517 nm in the visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolorized as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The water extract of *A. tricolor* leaves appeared to be as potent as vitamin C with a maximum inhibition of 62.96 % at 100 µg/mL, which is comparable to 67.64 % for vitamin C at the same concentration. Petroleum ether extracts having less inhibition than other extracts, isolated compound and vitamin C. The reducing ability of a compound generally depends on the presence of reductants, which exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom. The presence of reductants (*i.e*. antioxidants) in *A. tricolor* leaves extracts causes the reduction of the Fe^{3+} ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Fig. 5 shows the reductive capabilities of the *A. tricolor* leaves extracts compared to ascorbic acid. The reducing power of *A. tricolor* leaves extracts was very potent and the power of the extract was increased with quantity of sample. The water extract of *A. tricolor* leaves appeared to be as potent as vitamin C with a maximum absorbance of 0.383 at 100 µg/ mL, which was found to be comparable with 1.501 of vitamin C at the same concentration. Water extracts also showing more absorbance value compared to vitamin C in hydrogen peroxide scavenging activity. Two extracts and isolated compounds except the chloroform and petroleum ether with low antioxidant activity, have been considered for total antioxidant capacity estimation where in these were compared with vitamin C in different concentration like 100, 200, 300, 400 and 500 µg/mL. Even in this study the water extracts did show maximum absorbance compared to vitamin C. This study suggests that these plants possess antioxidant activities which can counteract the oxidative damage induced by the malaria parasite. This may be one of their modes of action in malaria therapy.

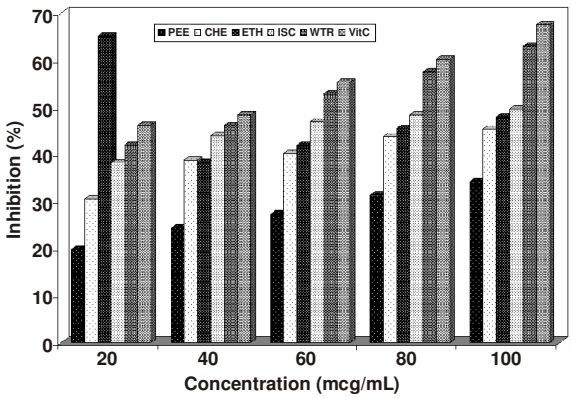


Fig. 4. DPPH radical scavenging activity of leaves extracts of *A. tricolor* and vitamin C; PEE = petroleum ether extract, CHE = Chloroform extract, ETH = Ethanolic, ISC = Isolated compound, WTR = Aqueous extract, VitC = Vitamin C

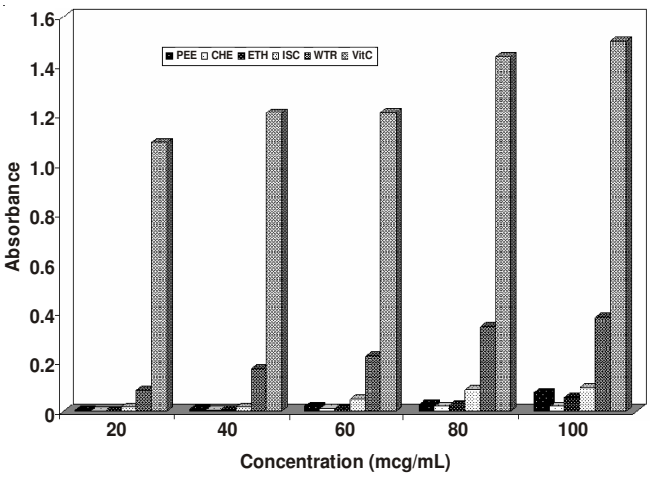


Fig. 5. Reducing power of leaves extracts of *A. tricolor* and vitamin C, PEE=petroleum ether extract, CHE = Chloroform extract, ETH = Ethanolic, ISC = Isolated compound, WTR = Aqueous extract, VitC = Vitamin C.

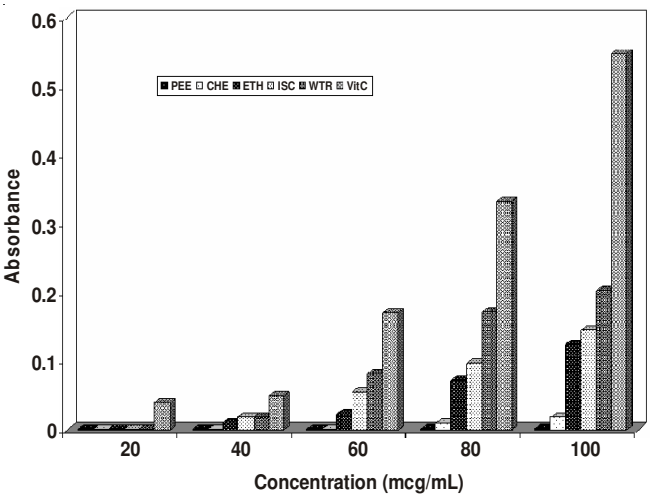


Fig. 6. Hydrogen peroxide scavenging activity of leaves extracts of *A. tricolor* and vitamin C; PEE=petroleum ether extract, CHE = Chloroform extract, ETH = Ethanolic, ISC = Isolated compound, WTR = Aqueous extract, VitC = Vitamin C.

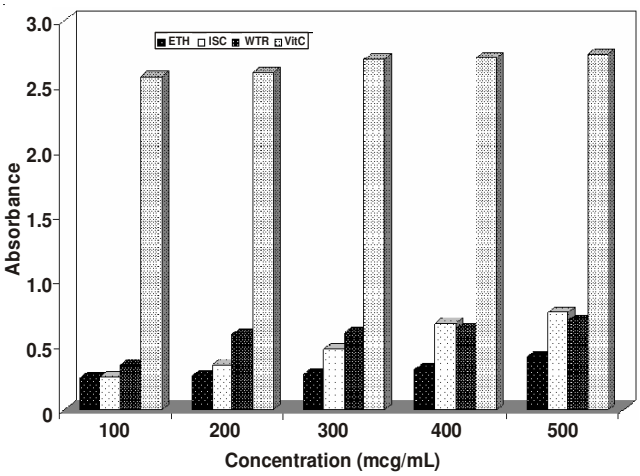


Fig. 7. Total antioxidant activity of leaves extracts of *A. tricolor* and vitamin C; ETH = Ethanolic, ISC = Isolated compound, WTR = Aqueous extract, VitC = Vitamin C

Conclusion

It may be concluded that the plant *A. tricolor* is endowed with significant antimicrobial and antioxidant activity due to the presence active constituents like phenolic compounds and others, there by justifying its use in the indigenous system of medicine.

REFERENCES

1. J. Swarbrick and J.C. Boylan, Plants as Drugs, Encylopedia of Pharmaceutical Technology, New York: Marcel Dekker Inc., edn. 2, Vol. 3, pp. 2214-2216 (2002).
2. K.M. Cheety, K. Sivaji and R.K. Tulasi, Flowering Plants of Chitoor District, Tirupati, India: Students Offset Printers, edn. 1, p. 291 (2008).
3. www.pfaf.org/database/plants.php?.
4. Plant Resources of South-East Asia No. 8: Vegetables, Wageningen, the Netherlands: Pudoc Scientific (1993).
5. http://www.tropilab.com/amaranth.html
6. S. Shukla, A. Bhargava, A. Chatterjee, J. Srivastava, N. Singh and S.P. Singh, Plant Foods for Human Nutrition (Formerly *Qualitas plantarum)*, Vol. 61, p. 21 (2006).
7. G. Jerz, T.N. Arrey, V. Wray, Q. Du and P. Winterhalter, *Innovat. Food Sci. Emerg. Technol.*, **8**, 413 (2007).
8. T. Fernando and G. Bean, *Food Chem*., **15**, 233 (1984).
9. M. Piatelli, M. Denicola and V. Castrogiovanni, *Phytochemistry*, **8**, 731 (1969).
10. A.K. Stobart, N.J. Pinfield and L.T. Kinsman, *Planta,* **94**, 152 (1970).
11. H. Wagner and S. Bladt, Plant Drug Analysis, Springer International Publication, edn. 2 (2004).
12. E. Stahl, Thinlayer Chromatography, Springer International Publication, edn. 2, pp. 686-705.
13. J.B. Harbone, Phytochemical Methods, Springer International Publication, edn. 3, pp. 74-83 (1998).
14. A. Kothari and N. Shrivasthava, *Indian Drugs*, **42**, 133 (2004).
15. Indian Pharmacopoeia, Ministry of Health, Government of India, New Delhi, Appendix 9, pp. 100-143 (1996).
16. Y. Okada and M. Okada, *J. Agric. Food Chem.*, **46**, 401 (1998).
17. G.C. Yen and P.D. Duh, *J. Agric. Food Chem*., **42**, 629 (1994).
18. M. Upadhye, A. Dhiman and A. Shirwaikar, *Int. J. Adv. Pharmacol. Toxicol.*, **10**, 127, (2009).
19. A. Sharma, S. Bharadwaj, A.S. Mann, A. Jain and M.D. Kharya, *Pharmacog. Rev.*, **1**, 232 (2007).
20. V. Sushela, V.K. Gopala Krishnan and Y.R. Hiranmai, *J. Adv. Pharmacol. Toxicol.*, **10**, 113 (2009).
21. M. Behari and R.K. Sharma, *Acta Cienc. Ind*., **17**, 357 (1991).
22. O. Polterait, *Curr. Org. Chem.*, **1**, 415 (1997).