An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

FREE RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF WHOLE PLANT OF *TRIANTHEMA TRIQUETRA* ROTTLER EX WILD: AN *IN-VITRO* EVALUATION

R. Indumathy*and Ajithadas Aruna[1]

Institute of Pharmacology, Madras Medical College, Chennai-600 003, India.

[1]Directorate of Medical Education, Chennai-600 010, India

ARTICLE INFO

Key Words

In-vitro antioxidant, DPPH radical activity, Superoxide radical scavenging activity, nitric oxide radical scavenging activity and hydroxyl radical activity.

ABSTRACT

Objective: To explore the *in-vitro* antioxidant potential of various extracts of whole plant of *Trianthema triquetra* by different *in-vitro* methods.

Methods: The various extracts (pet ether, chloroform, ethyl acetate and methanol) of *Trianthema triquetra* determined by DPPH activity, superoxide radical scavenging, nitric oxide radical scavenging activity and hydroxyl radical scavenging activity.

Results: An IC_{50} value was found that methanolic extract of *Trianthema triquetra* is more effective in free radical scavenging activity than that of other extracts. So, the *in-vitro* studies clearly showed that the methanolic extract of *Trianthema triquetra* has a significant antioxidant activity.

Conclusion: These *in-vitro* assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

INTRODUCTION

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electrons. and involved in many pathological conditions[1]. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defences or by supplementing with proven dietary antioxidants[2]. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in foods have side effect and are carcinogenic[3]. Plant polyphenolic compounds, such as flavonoids are described as scavengers of reactive oxygen species[4]. Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants has been extensively investigated[5]. Most sources of natural antioxidants originate from plant materials, but the content of polyphenolic compounds in the seeds and pericarp of tropical and subtropical flora has sparsely reported[6]. *Trianthema triquetra* (Aizoaceae) is a small prostrate, branched herb found almost throughout India. It is commonly known as Red spinach in English and siru sharunai in Tamil[7]. *T. triquetra* has been used in various parts of Arabia, Africa, Australia and India[8]. The stems and branches are slender usually red, leaves are small, succulent, flowers are several in axil, Fruit is a capsule of

2.3mmx1.5mm, 2-3 seeded compressed, orbicular reni form in black[9]. Leaves are used in hepatoprotective activity[10]. *Trianthema triquetra* contains various constituents like steroids, fatty acids, terpenoids, phenolics, flavonoids, gums, resins, quinones and saponins[11]. However, no data are available in the literature on the antioxidant activity of whole plant of *Trianthema triquetra*. Therefore we undertook the present investigation to examine the antioxidant activities of various extract of whole plant of *Trianthema triquetra* through different *in vitro* models.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The whole plant of *Trianthema triquetra*, were collected from Senkottai, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The plant material, were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[12] for 24hrs then marc was subjected to chloroform for 24hrs followed by ethyl acetate (76-78^{0}C) for 24hrs and then marc was subjected to methanol for 24hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in vacuum at 35° to 40°and dry powder was obtained.

Evaluation of Antioxidant activity by *in vitro* Techniques

DPPH photometric assay[13]

The effect of extract on DPPH radical was assayed using the method of Mensor *et al* (2001)[13]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactivity(\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard. The inhibitory concentration for reduction of 50% absorbance (IC50) was calculated using linear regression analysis. An average of triplicate reading was calculated.

Superoxide radical scavenging activity[14]

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[14]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5mM NBT) solution, 0.2ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12mM) and 2.5ml of phosphate buffer (0.067M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30min and the absorbance at 560nm was measured against the control samples. Quercetin was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Nitric oxide radical scavenging activity[15]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964)[15].The reaction mixture (3ml) containing 2ml of sodium nitroprusside (10mM), 0.5ml of phosphate buffer saline (1M) were incubated at 25^{0}C for 150mins. After incubation, 0.5ml of the reaction mixture containing nitrite was pipetted and mixed with 1ml of sulphanilic acid reagent (0.33%) and allowed to stand for

5min for completing diazotization. Then 1ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30mins. The absorbance was measured at 546nm. The percentage inhibition was obtained using the formula

$$Scavengingactivity(\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

and the graph was constructed by plotting concentration versus percentage inhibition and a linear regression equation calculated.

Hydroxyl radical scavenging activity[16]

This was assayed as described by Elizabeth and Rao (1990)[16]. The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate –EDTA –H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM), 0.1 ml EDTA (0.1mM), 0.1ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1ml KH_2PO_4-KOH buffer, P^H 7.4 (20mM) and various concentrations of plant extract in a final volume of 1ml treated with the reaction mixture was incubated for 1 hour at 37^0C. Deoxyribose degradation was measured as TBARs and the percentage inhibition was calculated. Rutin was used as standard. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

RESULTS AND DISCUSSION

Lot of interest has been shown towards plant derived antioxidants which have been employed in protecting bio-molecules against free radical mediated damages[17-19]. During the process of utilization of oxygen in normal physiological and metabolic processes, oxygen-derived free radicals are formed. The lipids, proteins and DNA are attacked by these free radicals and thus leading to several diseases. The free radicals may be neutralized by antioxidants[20]. *In vitro* screening methods are preliminary methods which show the way for *in vivo* evaluation studies[21]. Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases[22].They are also involved in autoimmune disorders like rheumatoid arthritis etc. The various extracts pet ether, chloroform, ethyl acetate and methanol extracts of the whole plants of *Trianthema triquetra* were subjected to *in vitro* antioxidant activity.

DPPH radical scavenging activity

The results obtained for the DPPH radical scavenging activity of the extracts of the whole plants of *Trianthema triquetra* are presented in Table 1. It was observed that the maximum scavenging activity at 1000µg/ml and IC_{50} value of petroleum ether extract was found to be 43.25% and 1175µg/ml, chloroform extract was found to be and 53.60% and 890µg/ml, ethyl acetate extract was found to be 65.58% and 425µg/ml and methanol extract it was found to be and 85.25% and 220µg/ml respectively, while for standard rutin, it was found to be 79.84% and 380µg/ml respectively. The IC50 value for methanol extract of *Trianthema triquetra* were significantly lower than that of standard which indicated that the extracts have very good free radical inhibition at lower concentration.

Inhibition of superoxide anion radical scavenging activity

The results obtained for superoxide anion radical scavenging potential of the extracts of the whole plants of *Trianthema triquetra* are presented in Table 2. It was observed that the maximum superoxide radical scavenging activity at 1000µg/ml and IC_{50} value of petroleum ether extract was found to be 33.25% and 1450µg/ml, for chloroform extract, it was found to be 49.55% and 1025µg/ml, for ethyl acetate extract it was found to be 53.78% and 490µg/ml while for methanol extract of *Trianthema triquetra* it was found to be 66.25% and 360 µg/ml respectively, while for standard Rutin, it was found to be 98.13% and 80µg/ml respectively.

Table 1: DPPH scavenging potential of various extracts of Whole plants of *Trianthema triquetra*

S. No	Conc. in µg/ml	% scavenging ± SEM*				
		Petroleum ether Extract	Chloroform extract	Ethyl acetate extract	Methanolic extract	Standard (Rutin)
1	125	25.02 ± 0.48	33.07 ± 0.04	37.56 ± 0.99	46.05 ± 0.78	17.75 ± 0.45
2	250	26.36 ± 0.01	37.71 ± 0.29	46.05 ± 0.78	61.17 ± 0.35	59.95 ± 0.82
3	500	39.04 ± 0.08	45.36 ± 0.02	59.25 ± 0.61	85.36 ± 0.25	63.73 ± 0.20
4	1000	43.25 ± 0.04	53.60 ± 0.79	62.58 ± 0.83	86.25 ± 0.01	79.84 ± 0.19
IC_{50} (µg/ml)		**1175**	**890**	**425**	**220**	**380**

***All values are expressed as mean ± SEM for three determinations**

Table 2: Superoxide anion radical scavenging potential of various extracts of whole plants of *Trianthema triquetra*

S. No	Conc. in µg/ml	% inhibition ± SEM*				
		Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanolic extract	Standard (Quercetin)
1	125	20.18 ± 0.09	11.57 ± 0.44	24.74 ± 0.04	33.45 ± 0.39	68.85 ± 0.66
2	250	25.67 ± 0.02	33.67 ± 0.78	42.66 ± 0.60	38.47 ± 0.18	74.81± 1.09
3	500	31.02 ± 0.90	36.17 ± 0.11	51.26 ± 0.98	63.01 ± 0.05	92.90 ± 0.11
4	1000	33.25 ± 0.94	49.55 ± 0.01	53.78 ± 0.12	66.25 ± 0.04	98.13 ± 1.92
IC_{50} in µg/ml		**1450**	**1025**	**490**	**360**	**80**

***All values are expressed as mean ± SEM for three determinations**

Table 3: Nitric oxide anion radical scavenging potential of various extracts of whole plants of *Trianthema triquetra*

S. No	Conc. in µg/ml	% inhibition ± SEM*				
		Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanolic extract	Standard (Ascorbic acid)
1	125	18.77 ± 0.07	23.87 ± 0.88	19.88 ± 0.80	52.37 ± 0.01	26.87 ± 0.09
2	250	28.47 ± 0.02	33.77 ± 0.74	33.57 ± 0.01	58.57 ± 0.01	40.38 ± 0.08
3	500	33.58 ± 0.03	42.57 ± 0.12	49.67 ± 0.70	63.37 ± 0.08	71.64 ± 0.43
4	1000	41.89 ± 0.01	48.68 ± 0.73	55.87 ± 0.76	62.87 ± 0.09	75.23 ± 0.02
IC_{50} (µg/ml)		**1450**	**1050**	**510**	**105**	**280**

***All values are expressed as mean ± SEM for three determinations**

Table 4: Hydroxyl radical scavenging potential of various extracts of whole plants of *Trianthema triquetra*

S. No	Conc. in µg/ml	% inhibition ± SEM*				
		Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanolic extract	Standard (Rutin)
1	125	12.78 ± 0.03	15.74 ± 0.09	19.37 ± 0.04	33.67 ± 0.03	26.87 ± 0.09
2	250	19.57 ± 0.01	25.37 ± 0.06	33.57 ± 0.93	48.65 ± 0.31	40.38 ± 0.08
3	500	23.22 ± 0.92	28.57 ± 0.12	48.36 ± 0.22	67.27 ± 0.01	71.64 ± 0.43
4	1000	28.70 ±0.63	33.25 ± 0.35	57.25 ± 0.02	72.57 ± 0.32	75.23 ± 0.02
IC_{50} (µg/ml)		**1575**	**1370**	**595**	**280**	**280**

***All values are expressed as mean ± SEM for three determinations**

The IC_{50} value for methanol extract of *Trianthema triquetra* was significantly higher than standard and possess to inhibiting superoxide radical.

Nitric oxide radical scavenging activity

The results obtained for nitric oxide radical scavenging potential of the extracts of the whole plants of *Trianthema triquetra* are presented in Table 3. It was observed that the maximum of nitric oxide radical scavenging activity at 1000µg/ml and IC_{50} value of petroleum ether extract of *Trianthema triquetra* was found to be 41.89% and 1450µg/ml, chloroform extract was found to be 48.68% and 1050µg/ml, for ethyl acetate extract, it was found to be 55.87% and 510µg/ml and for methanol extract, it was found to be 62.87% and 105 µg/ml respectively while for standard quercetin was found to be 75.23% and 280µg/ml respectively. The IC_{50} value for methanol extract of *Trianthema triquetra* were significantly lower than that of standard which indicated that the extracts have very good free radical inhibition at lower concentration.

Hydroxyl radical scavenging activity

The results obtained for hydroxyl radical scavenging activity of the extracts of the whole plants of *Trianthema triquetra* are presented in Table 4. It was observed that the maximum of hydroxyl radical scavenging activity at 1000µg/ml and IC_{50} value of petroleum ether extract of *Trianthema triquetra* was found to be 28.70% and 1575µg/ml, chloroform extract of exhibited 33.25% inhibition and an IC_{50} value of 1370µg/ml while for ethyl acetate extract, it was found to be 57.25% and 595µg/ml and for methanol extract it was found to be 72.57% and 280µg/ml respectively, while for that of standard Rutin was found to be 75.23% and 280 µg/ml. Hydrogen peroxide scavenging of extracts may be due to phenolic and flavonoids present in the extracts which reduces H_2O_2 to water by donating electron.

CONCLUSION

The present study was clearly indicated the methanolic extract of *Trianthema triquetra* showed strong antioxidant activity when compared with other extracts. The antioxidant activity of the extract may be attributed to the phenolic compounds and flavonoids with potential application to reduce oxidative stress with health benefits[23]. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

REFERENCES

1. Madhavi DL, Deshpande SS, & Sulunkhe DK. Food antioxidants: technological, toxicological and health perspectives; New york: Marcel dekker. 1996.
2. Stanner SA, Hughes J, Kelly CN, Buttriss JA. Review of the epidemiological evidence for the 'antioxidant hypothesis. Public Health Nutrition; 2000, 7: 401-422.
3. Branen AL. Toxicological and biochemistry of butylated hydroxyl anisole and butylated hydroxytoluene. J of American oil chemists society; 1975, 52: 59-63.
4. Chen S, Hwang J, Deng PSK. Inhibition of NAD(P)H: Quinone acceptor oxidoreductase by flavones: a structural activity study. Archives of biochemistry and biophysics; 1993, 303: 72-77.
5. Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. Methods in enzymology; 1994, 234: 279-293.
6. Elizabeth M, Williamson. Textbook of Major herbs of Ayurveda. (I); 2002: 7-11.
7. Neueschriften Get. Naturf. Freunde Berlin, *Trianthema Triquetra* Rottler Ex. Willd Flora Base: The Western Australian flora.1803;4:181.
8. Forssk Vahl. Australian plant census, *Published in:* Black, J.M. 1948, Flora of South Australia Edn. 2.2 255-521.
9. Australian Plant Census. Published in: Eichler, Hj.1965, Supplement to J.M. Black's Flora of South Australia (Second Edition, 1943-195

10. Australian Plant Census. *Trianthema glaucifolia* F. Muell. *Published in:* Hartmann, H.E.K., Meve, U. & Liede-Schumann, S. 2011,Towards a revision of Trianthema, the Cinderella of Aizoaceae. Plant Ecology and Evolution. 144(2):177-21.

11. Mansoor ahmeed , Muhammad ashraf, F. Al-Quriany, Tahira Nawaz. Medicinal flora of the Cholistan desert: a review Pak. J Bot. 2011; 43:39-50

12. Harborne JB. Phytochemical methods 11Edn In Chapman &, Hall. New York: 1984; 4-5.

13. Mensor LL, Meneze FS, Leitao GG, Reis AS, *et al*., Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res.2001;15: 127-130.

14. Winterbourne CC, Hawkins RE, Brain M and Carrel RW. The estimation of red cell superoxide dismutase activity. J. Lab.chem.Med. 1975;85: 337-341.

15. Garrat DC.The quantitative analysis of drugs, Champman and Hall, Japan.1964;3:456-458.

16. Elizabeth K and Rao MNA. Oxygen radical scavenging activity of curcumin, Int.J.Pharm.1990; 58:237-240.

17. Larson RA. The antioxidants of higher plants. Phytochem.1998; 27(4):969-978.

18. Gazzani G, Papetti A, Massolini G and Daglia M. Anti and pro-oxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. J Agric Food Chem.1988; 46: 4118-4122.

19. Velioglu YS, Mazza G, Gao L and Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem.1988; 46: 4113-4117.

20. Jayprakash GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed extracts on peroxidation models *in vitro*. J Agric Food Chem. 2011; 55:1018-1022.

21. Elayaraja A, Vijayalakshmi M, and Devalarao G. *In vitro* free radical scavenging activity of various root and rhizome extracts of *acorus calamus* linn. Int J Pharma Bio Sci. 2010;4:1.

22. Andlauer W and Furst P. Antioxidative power of phytochemicals with special reference to cereals. Cereal Foods World.1998; 43:356-359.

23. Indumathy R and Ajithadas Aruna. Free Radical Scavenging activities, Total Phenolic and Flavonoid content of *Lepidium sativum* (Linn.) International Journal of Pharmacy and Pharmaceutical Sciences.2013; 5(4).