ISSN: 0975-766X

Available Online through
www.ijptonline.com

Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT OF TRIUMFETTA RHOMBOIDEA

P. Sivakumar[1], R. Sambath Kumar*[1], T. Sivakumar[1], P. Perumal[1], B. Jayakar[2].

1. Department of Pharmaceutical Chemistry, Natural Product Research Laboratory, J. K. K. Nataraja College of Pharmacy, Komarapalayam 638 183, Namakkal, Tamilnadu, India.
2. Department of Pharmaceutical Chemistry, College of pharmacy, Vinayaka missions University, NH-47, Sankari Main Road, Ariyanoor and Salem-636308, TN, India.

Email:*sambathju2002@yahoo.co.in*

Received on 25-07-2010 ***Accepted on 08-08-2010***

Abstract:

Triumfetta rhomboidea (Family: Tiliaceae) is being used in traditional medicine for the treatment of tumors, gonorrhea and leprosy. Ethanol extract of its leaves were subjected for *in vitro* antioxidant activity using four different methods such as , 1- diphenylpicryl- hydrazyl radical (DPPH), nitric oxide radical and 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) ABTS radical cation assay and H_2O_2 radical. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner in all the models. The antioxidant activities were compared to standard antioxidants such as ascorbic acid and rutin. The ethanol extract of *Triumfetta rhomboidea* (EETR) exhibited potent DPPH and ABTS radical scavenging activity with IC_{50} values 16.56 and 39.00 µg/ml, respectively. The EETR showed significant antioxidant activity against H_2O_2 radical with IC_{50} values 97.80 µg/ml and moderate antioxidant activity against nitric oxide radical with IC_{50} value 345.50 µg/ml, respectively. Based on the results it can be concluded that ethanol extract of *Triumfetta rhomboidea*, a natural herb may have potential antioxidant effects against several oxidants.

Keywords: *Triumfetta rhomboidea*; Tiliaceae; Antioxidants; Free radical scavengers.

INTRODUCTION

Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), singlet oxygen ($^1O_2$) and non-free radical species such as hydrogen peroxide (H_2O_2) are various forms of activated oxygen and often generated by oxidation product of biological reactions or exogenous factors (Ceruitti, 1991; Yıldırım et al., 2001). In living organism, various ROS can form by different ways. Normal aerobic respiration stimulates polymorph nuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of ROS include tobacco smoke, certain pollutants, organic solvents, and pesticides (Davies, 1994). In addition, it is well known that ROS induce some oxidative damage to biomolecules like lipids, nucleic acids, proteins, amines, deoxyribonucleic acid and carbohydrates. Its damage causes ageing, cancer, heart disease, stroke, arteriosclerosis, diabetes and other many diseases (Kehrer, 1993). ROS are continuously produced during normal physiologic events, and removed by antioxidant defense mechanisms (Halliwell et al., 1992a). There is a balance between generation of ROS and antioxidant system in organisms. In pathological condition, ROS are overproduced and result in lipid peroxidation and oxidative stress. The imbalance between ROS and antioxidant defense mechanisms leads to oxidative modification in cellular membrane or intracellular molecules. Various endogenous antioxidant defense mechanisms play an important role in the elimination of ROS and lipid peroxides, and therefore, protect the cells against toxic effects of ROS and lipid peroxides (El-Habit et al., 2000).

Many antioxidant compounds, naturally occurring from plant sources, have been identified as free radical or active oxygen scavengers (Duh, 1998). Recently, interest has increased considerably in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity. Natural antioxidants can protect the

human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods (Lai et al., 2001; Munir Oktay et al., 2003).

The plant *Triumfetta rhomboidea* (Family: Tiliaceae) is distributed throughout tropical and subtropical part of India, and also found in Ceylon, Malay, Africa and America. All parts of the plant are used in natural medicine in the tropics. Roots of *Triumfetta rhomboidea* have been traditionally used in dysentery, intestinal ulcer and as diuretic. Leaves and stem are used in treatment of tumors, gonorrhoea and leprosy (Kirtikar et al., 1975; Nadkarni, 1994; Khare, 2007). However, no reports are available to show the antioxidant property of the plant. Hence, the present study has been undertaken to investigate the *in vitro* antioxidant activity of the ethanol extract of leaves of *Triumfetta rhomboidea* (EETR) using different *in vitro* models.

MATERIALS AND METHODS

Plant material and Extraction

The leaves of *Triumfetta rhomboidea* was collected from Yercaud, Salem district, Tamilnadu India. The collected plant was authenticated at Botanical survey of India, Coimbatore. The shade dried leaves was powdered and extracted using ethanol as solvent in a Soxhlet apparatus and after complete extraction (48 h) the solvent was removed by distillation under reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield (12.0% w/w) a solid residue (Harbone, 2005).

Chemicals

2, 2-diphenyl-1-picryl hydrazyl (DPPH) and 2, 2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) diammonium salt were obtained from Sigma Aldrich Co, St Louis, USA. Ascorbic acid and rutin were obtained from S.D. Fine Chem., Biosar, India. Hydrogen peroxide (30%) was obtained from Qualigen Fine Chemicals, Mumbai, India. All chemicals and solvents were of analytical grade, obtained from SRL and Merck, Mumbai, India.

IN-VITRO ANTIOXIDANT ASSAY

DPPH radical scavenging activity

The antioxidant activity of leaf extract of *Triumfetta rhomboidea* and the standard compounds were assessed on the basis of radical scavenging effect on the DPPH stable free radical (Shreejayan et al., 1996; Hwang et al., 2001). The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of various concentrations of the extract or the standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37ºC for 30 min. Absorbance was measured at 517 nm using ELISA reader. The percentage scavenging activity was calculated by comparing the result of the control (methanol and 1ml DPPH) using the formula

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD control - OD sample}}{\text{OD control}} \times 100$$

IC_{50}, which is the concentration of the sample required to scavenge 50% of free radicals was calculated.

Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the modified Griess Ilosvog reaction (Garrat, 1964; Badami et al., 2003). The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (1 ml) and extracts or standard solutions (1 ml) was incubated at 25^{o}C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was removed, 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed and allowed to stand for 5 min for completing diazotization, and 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min. A pink coloured chromophore was formed in diffused light. The absorbance was measured at 540 nm against the corresponding blank solutions in microtiter plates using ELISA reader. IC50 values were calculated.

Scavenging of ABTS radical cation

In this improved version, ABTS- the oxidant is generated by persulfate oxidation of 2,2- azinobis (3-ethyl benzothiazoline-6-sulfonic acid) diammonic salt. ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS solution (2mm) with 17mm, 0.3ml potassium persulfate and the mixture were allowed to stand in dark at room temperature for 12-16 h before use. For the study, different concentration (31.25-500 µg/ml) of ethanol extract (0.5ml) were added to 0.2ml of various concentrations of the extract or standards, 1.0ml of distilled DMSO and 0.16ml of ABTS solution was added to make a final volume of 1.36ml. The percentage inhibition was calculated (Guddadarangavvanahally et al., 2004; Jayaprakasha et al., 2004; Halliwell., 1994).

Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1 ml of the extract or standard in ethanol were added to 2 ml of hydrogen peroxide solution in PBS. The absorbance was measured at 230 nm after 10 min against a blank solution that contained extract or standard in PBS without hydrogen peroxide. IC_{50} values were calculated (Guddadarangavvanahally et al., 2004; Jayaprakasha et al., 2004).

RESULTS

The ethanol extract of *Triumfetta rhomboidea* was also evaluated for their *in vitro* antioxidant activity using four different methods such as , 1- diphenylpicryl- hydrazyl radical (DPPH), nitric oxide radical, 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) ABTS radical cation assay and H_2O_2 radical. The EETR was found to be more active in most of the methods tested. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner in all the models. The ethanol extract of *Triumfetta rhomboidea* exhibited potent DPPH and ABTS radical scavenging activity with IC_{50} values 16.56 ± 0.88 and 39.00 ± 1.31µg/ml, respectively. EETR showed significant antioxidant activity against H_2O_2 radical with IC_{50} values 97.80 ± 4.55 µg/ml and moderate antioxidant activity against nitric oxide radical with IC_{50} value

345.50 ± 11.00 µg/ml, respectively. All the experimental values were compared with standard ascorbic acid and rutin. Among the four methods, EETR was found to be more active in inhibiting H_2O_2 free radical with IC_{50} values 97.80 ± 4.55 µg/ml and comparable with positive controls, ascorbic acid and rutin having IC_{50} as 187.33 and 63.66 µg/ml respectively. It produced nearly 84.56% of inhibition of H_2O_2 radicals, seems to be a good antioxidant agent. These details at various concentrations for different methods are given in the Table-1. From these results, EETR was found to possess comparable free radical scavenging activity as that of standard ascorbic acid and rutin in all the models.

Table 1. *In vitro* antioxidant activity of ethanol extract of *Triumfetta rhomboidea* (EETR) against DPPH, Nitric oxide, ABTS, H_2O_2 radicals.

Extract / Standards	IC_{50} values ± SEM µg/ml* by methods							
	DPPH		Nitric oxide		ABTS		H_2O_2	
	Conc µg/ml	% Inhibition	Conc µg/ml	% Inhibition	Conc µg/ml	% Inhibition	Conc µg/ml	% Inhibition
Ethanol extract of *Triumfetta rhomboidea* EETR	125	75.00±2.66	500	65.80±2.50	125	83.56±1.05	500	84.56±0.98
	62.5	64.25±1.25	250	40.15±1.25	62.5	75.16±3.15	250	78.00±2.75
	30.00	59.50±1.50	125	31.25±3.56	30.00	40.50±2.21	125	71.56±1.05
	15.03	47.35±1.35	62.5	7.80±0.65	15.03	27.56±1.30	62.5	31.00±1.15
	7.50	17.88±0.85	31.25	00	7.51	19.50±1.65	31.25	6.50±0.35
IC_{50} µg/ml	16.56 ± 0.88		345.50 ±11.00		39.00 ± 1.31		97.80 ± 4.55	
Standards								
Ascorbic acid	2.69 ± 0.05		---		11.25 ± 0.49		187.33 ± 1.93	
Rutin	---		65.44 ± 2.56		0.51± 0.01		36.66 ± 0.22	

*Average of three determinations, values was mean ± S.E.M.

DISCUSSION

Lipid peroxidation has gained more importance nowadays because of its involvement in the pathogenesis of many diseases like atherosclerosis, cancer, diabetes mellitus, myocardial infarction, immunological incompetence, neurodegenerative disorders and also in aging. Protective role of antioxidants against free radical mediated toxicity is now well established. Several plant extracts and phytoconstituents have shown potent antioxidant activity (Halliwell, 1994b).

In the present study, the ethanol extract of *Triumfetta rhomboidea* was studied for *in vitro* antioxidant activity using four standard methods. EETR exhibited potent antioxidant activity in DPPH and ABTS, and H_2O_2 radical methods and moderate antioxidant activity against nitric oxide radical. The variations in activity may be due to the fact that diversity in the basic chemical structure of phytoconstituents possesses different degree of antioxidant activity against different free radicals. The preliminary phytochemical investigation revealed the presence of flavonoids, steroids, saponins and terpenoids compounds in ethanol extracts of the plant. Plant flavonoids are known to exhibit potent antioxidant activity. Hence, the observed antioxidant activity of the extracts of *Triumfetta rhomboidea* may be due to the presence of these constituents. The above observation reveals that the ethanol extract of *Triumfetta rhomboidea*, a natural herb may have potential antioxidant effects against several oxidants.

REFERENCES

1. Badami S, Mahesh KG, Suresh B. Antioxidant activity of the ethanolic extract of Striga *orobanchioides*. *J. Ethnopharmacol*, 2003, 85, 227- 230.

2. Ceruitti PA. Oxidant stress and carcinogenesis. *Europan J. Clinic. Investigation*, (1991) 21, 1-11.

3. Davies KJA. Oxidative stress: The paradox of aerobic life. *Biochemis. Society Symphosium*, (1994) 61, 1–34.

4. Duh PD. Antioxidant activity of burdock (*Arctium lappa* Linn): Its scavenging effect on free radical and active oxygen. *J. American Oil Chemist's Society*, (1998) 75, 455–465.

5. El-Habit OHM, Saada HN, & Azab KS. The modifying effect of ß-carotene on gamma radiation-induced elevation of oxidative reactions and genotoxicity in male rats. *Mutation Research*, (2000) 466, 179–186.

6. Garrat DC. The Quantitative Analysis of Drugs. 3rd Ed., Chapman and Hall Ltd., Japan, (1964)456-458.

7. Guddadarangavvanahally KJ, Lingamallu JR, Kunnumpurathu KS. Antioxidant activities of flavidin in different *in vitro* model systems. *Bioorg. Med. Chem*, (2004)12, 5141-5147.

8. Halliwell B, Gutteridge JMC, & Cross CE. Free radicals, antioxidants, and human disease: Where are we now. *J. Laboratory and Clinic Medical*, (1992) 119, 598–620.

9. Halliwell B. Free radicals and antioxidants: A personal view. *Nutrition Reviews*, (1994) 52, 253-265.

10. Harbone JB. In; Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis., 3rd edition. Springer (India) Pvt. Ltd., New Delhi, (2005) 5-16, 22.

11. Hwang BY, Kim HS, Lee JH, Hong YS, Lee KS, Lee JJ. Antioxidant activity of benzoylated flavon-3-ol glycosides from *Celastrus orbiculatus. J. Nat. Prod*, (2001) 64, 82- 84.

12. Jayaprakasha GK, Jaganmohan Rao L, Sakariah KK. Antioxidant activities of flavidin in different in-vitro model systems. *Bioorg. Med . Chem*, (2004)12, 5141-5146.

13. Kehrer JP. Free radicals as mediators of tissue injury and disease. CRC Critical Review Toxicology, (1993) 23, 21–48.

14. Khare CP. In; Indian Medicinal Plants, an Illustrated Dictionary, Rajkamal Electric Press, New Delhi, (2007) 677.

15. Kirtikar KR, Basu BD. Indian Medicinal Plant, 2nd edition, Vol-I, Bishen Sing Mahendra Pal Sing, Dehradun, (1975) 395-396.

16. Lai LS, Chou ST, & Chao WW. Studies on the antioxidative activities of hsian-tsao (Mesona procumbens Hemsl) leaf gum. Jour. Agricultural and Food Chemistry, (2001) 49, 963–968.

17. Marcocci L, Packer L, Sckaki A, Albert GM. Antioxidant action of *Ginkg biloba* extracts EGb 766. *Methods Enzymol*, (1994) 234, 462-475.

18. Munir Oktay., Ilhami Gulcin., Irfan Kufrevioglu O. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *Lebensm. Wiss. U. Technol,* (2003) 36, 263–271.

19. Nadkarni AK. Dr. K.M. Nadkarni's Indian Materia Medica, 3rd revised edition. Vol-1, Popular Prakashan Pvt. Ltd., Mumbai, (1994), 1251.

20. Re R, Pellegrini M, Proteggente A, Pannala A, Yang M, Rice EC. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. *Free Radical Biol. Med*, 1999, 26, 1231-1237.

21. Shreejayan M, Rao MNA. Free radical scavenging activity of curcuminoids. *Arzneim-Forsch. Drug Res*, 1996, 46, 169-171.

22. Yıldırım A, Mavi A, & Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *J. Agricultural and Food Chemis*, 2001, 49, 4083–4089.

Corresponding author*

R. Sambath Kumar*

Email:*sambathju2002@yahoo.co.in*