ORIGINAL ARTICLE

Antioxidant activity of the medicinal plant *Enicostemma littorale* Blume

P. Abirami, M. Gomathinayagam, R. Panneerselvam
Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu, India

Medicinal plants are the source for wide variety of natural antioxidants. In the study reported here, we have conducted a comparative study between the different parts of the plant *Enicostemma littorale.* The amount of total phenols and antioxidant enzymes Glutathione-S-Transferase, Superoxide Dismutase, Catalase and Peroxidase activities were evaluated and also the non-enzymatic antioxidants ascorbic acid, α- tocopherol and Glutathione activities were evaluated. The results showed that the antioxidant activities varied greatly among the different plant parts used in this study and some parts are rich in natural antioxidants especially the flowers of *E. littorale*. These results suggest that *Enicostemma littorale* have strong antioxidant potential. Further study is necessary for isolation and characterization of antioxidant agents, which can be used to treat various oxidative stress-related diseases.

Key words: Antioxidant, enzymatic antioxidant, non-enzymatic antioxidant, total phenol

INTRODUCTION

Antioxidant help the organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Reactive oxygen species (ROS) formed *in vivo,* such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damage transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase but due to over-production of reactive species, induced by exposure to external oxidant substances or a failure in the defense mechanism, damage to cell structure, DNA, lipids and proteins.[1] Free radicals play an important role in some pathogenesis of serious diseases, such as neurodegenerative disorders, cancer, liver cirrhosis, cardiovascular diseases, atherosclerosis, cataracts, diabetes and inflammation.[2] *Enicostemma littorale* Blume (White Head) is a perennial glabrous medicinal herb (Gentianaceae). It is found distributed throughout the greater part of India and common in coastal areas. The plant is pungent and very bitter, antihelmintic, cures fever and vata diseases. It is also used as stomachic, laxative, antidiabetic, and crushed plant material is applied to snake-bites.[3] It contains phenols, tannins, flavonoids, glycosides, anthroquinones, sterols,[4] gentianine and swertiamarin.[5,6]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.94359

MATERIALS AND METHODS

Plant Collection

The fresh plants were harvested from Annamalai University experimental garden. The taxonomic identity of the plant was confirmed by the Botanist of Annamalai University, Tamil Nadu. The plant materials were washed under tap water and separated into leaves, flowers, stems and roots. The separated parts were cut into small pieces and then used for experimental studies.

Ascorbic acid

The ascorbic acid content was assayed as described by Omaye *et al.*,[7] One gram of fresh material was ground in a pestle and mortar with 5 ml of 10% TCA, the extract was centrifuged at 3500 rpm for 20 minutes. The pellet was re-extracted twice with 10% TCA and supernatant was increased 10 mL and used for estimation. To 0.5 mL of the extract, 1 mL of DTC reagent (2, 4-Dinitrophenyl hydrazine-Thiourea-$CuSO_4$ reagent) was added and mixed thoroughly. The tubes were incubated at 37°C for 3 hours and to this a solution of 0.75 mL of ice cold 65% H_2SO_4 was added. The tubes were then allowed to stand at 30°C for 30 min. The resulting colour was read at 520 nm in a spectrophotometer. The ascorbic acid

Address for correspondence: Dr. M. Gomathinayagam, Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu - 608 002, India. E-mail: gomsnayagam@yahoo.com
Received: 16-12-2011; **Accepted:** 27-01-2012

content was determined using a standard curve prepared with ascorbic acid and the results were expressed in milligrams per gram fresh weight.

Total phenols

Total phenolic content was assayed as described by Malick and Singh[8]; 500 mg of fresh plant tissue was ground in a pestle and mortar with 10 ml of 80 percent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated to dryness. The residue was dissolved with 5 ml of distilled water and used as extract. To 2 ml of the extract, 0.5 ml of Folin - Ciocalteu's reagent was added. After 3 min, 2 ml of 20% Na_2CO_3 solution was mixed thoroughly. The mixture was kept in boiling water for exactly one min. and after cooling the absorbance was read at 650 nm. The total phenol was determined using a standard curve prepared with different concentrations of gallic acid and expressed in mg g^{-1} dry weight.

α-Tocopherol

α-tocopherol activity was assayed as described by Backer *et al.*,[9] 500 mg of fresh tissue was homogenized with 10 mL of a mixture of petroleum ether and ethanol (2:1.6 v/v) and the extract was centrifuged at 10,000 rpm for 20 min and the supernatant was increased the used for estimation of α- tocopherol. To 1 mL of extract, 0.2 mL of 2% 2,2-dipyridyl in ethanol was added and mixed thoroughly and kept in the dark for 5 min. The resulting red colour was diluted with 4 mL of distilled water and mixed well. The resulting colour in the aqueous layer was measured at 520 nm. The α -tocopherol content was calculated using a standard graph made with a known amount of α-tocopherol. The results were expressed in milligrams per gram fresh weight.

Reduced glutathione

The reduced glutathione (GSH) content was assayed as described by Griffith and Meister[10] (1979); 200 mg of fresh material was ground with 2 ml of 2% metaphosphoric acid and centrifuged at 17,000 rpm for 10 min. Adding 0.6 mL 10% sodium citrate neutralized the supernatant. One millilitre of assay mixture was prepared by adding 100 µl extract, 100 µl distilled water, 100 µl of 6 mM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) and 700 µl of 0.3 mM NADPH. The mixture was stabilized at 25°C for 3–4 min. Then 10 µl of glutathione reductase was added and read the absorbance at 412 nm in spectrophotometer and expressed reduced glutathione content in µg g^{-1} fresh weight (FW).

Glutathione-S-transferase

Glutathione-S-transferase (GST) activity was assayed as described by Habig *et al.*,[11] 500 mg of fresh tissue was homogenized with 5 mL of sodium phosphate buffer. The homogenates were centrifuged at 5000 rpm for 10 min. The reaction mixture contained 0.1 ml of GSH, 0.1 ml of 1-chloro-2,4-dinitrobenzene and phosphate in total volume of 2.9 ml. The reaction was initiated by the addition of 0.1 ml of the enzyme extract. The readings were recorded every 15 seconds at 340 nm against distilled water blank for a minimum of three minutes in a spectrophotometer. GST activity was calculated as n mol conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 and 8.5 for chlorodinitrobenzene and dichloronitrobenzene, respectively. Protein was determined according to Lowry *et al.*,[12] using bovine serum albumin as standard.

Superoxide dismutase

Crude enzyme extract was prepared, for the assay of Superoxide dismutase (SOD) using the method of Hwang *et al.*;[13] 1 g of fresh tissue was homogenized with 10 mL of ice cold 50 mM sodium phosphate buffer containing 1 mM phenyl methyl sulfonyl fluoride (PMSF). The extract was filtered through a double layered cheese cloth. The extract was centrifuged at 12,500 rpm for 20 min at 4°C. The supernatant was saved and increased to 10 mL with the extraction buffer and used for the estimation of the SOD enzyme activity. The enzyme protein was determined by the Bradford[14] method. Superoxide dismutase activity was assayed as described by Beauchamp and Fridovich.[15] The reaction medium was prepared and to 3 mL reaction medium, 1 mL of enzyme extract was added. The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} potassium cyanide and 5.6×10^{-5} M nitroblue tetrasodium salt, dissolved in a 0.05 M sodium phosphate buffer (pH 7.8). The mixture was illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes. Illumination started to initiate the reaction at 30°C for 1 h. Those without illumination were saved as blank and kept in the dark. The absorbance was read at 560 nm in the spectrophotometer against the blank. Superoxide dismutase activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 per hour per milligram protein under the assay condition.

Catalase

Catalase (CAT) activity was assayed as described by Chandlee and Scandalios[16] 500 mg of frozen material was homogenized in 5 mL of ice cold 50 mM sodium phosphate buffer (pH 7.5) containing in 1 mM PMSF. The extract was centrifuged at 4°C for 20 min at 12,500 rpm. The supernatant was used for the enzyme assay. The assay mixture contained 2.6 mL of 50 mL of 50 mM potassium phosphate buffer (pH 7.0) 0.4 mL, 15 mM H_2O_2 and 0.04 mL of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in units of 1 mM of H_2O_2 reduction per minute per mg protein.

Peroxidase

Peroxidase (POX) activity was assayed as described by Reddy *et al.*,[17] 500 mg of frozen material was homogenized in 5 mL of 0.1 M phosphate buffer (pH 6.5) from the various parts of the plant, clarified by centrifugation and the supernatant was used for the assay. To 3.0 ml of pyrogallol solution, 0.1 ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5 ml of H_2O_2 was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer. One unit of peroxidase is defined as the change in absorbance/minute at 430 nm.

Statistical Analysis

Data were subjected to statistical analysis using statistical software package SPSS version 14 (SPSS Inc, Chicago, USA). One way analysis of variance (ANOVA) followed by Duncan multiple range test were employed and the differences between individual means were deemed to be significant at $P<0.05$.

RESULTS

Determination of Total Phenols

The total phenolic content is reported as gallic acid equivalents by reference to standard curve. Flowers had the highest total phenolic content (7.896 mg of GAE/g dw) followed by stem (5.893±0.01 mg of GAE/g dw)), leaf (5.582±0.06 mg of GAE/g dw)) and root (4.687±0.11 mg of GAE/g dw)), respectively.

Antioxidant Activities

The antioxidant activity from four parts of *Enicostemma littorale* (leaves, stems, roots and flowers) was evaluated. The ascorbic acid activity was found to be significantly higher ($P<0.05$) in leaves (4.416±0.05 mg g^{-1} FW) followed by flowers (4.108±0.34 mg g^{-1} FW), stem (3.608±0.04 mg g^{-1} FW) and then roots (0.366±0.26 mg g^{-1} FW). α-tocopherol activity was also found to be significantly higher ($P<0.05$) in leaves (2.659±0.07 mg g^{-1} FW) followed by flowers (2.511±0.11 mg g^{-1} FW), stem (2.482±0.02 mg g^{-1} FW) and then roots (1.512±0.05 mg g^{-1} FW). GSH activity was recorded to be significantly higher ($P<0.05$) in leaves (2.974±0.02µg g^{-1}FW) followed by flowers (2.562± 0.02 µg g^{-1}FW), stem (2.305±0.04 µg g^{-1}FW) and then roots (2.174±0.02 µg g^{-1}FW) [Figure 1]. For the enzymatic antioxidant properties, Glutathione –S-Transferase activity as found to be significantly higher ($P<0.05$) in flowers (8.456±0.02 n mol/mg protein) than in stem (8.413±0.02 n mol/mg protein) and leaves (8.052±0.04 n mol/mg protein) and roots (5.409± 0.05 n mol/mg protein). SOD activity was also found to be significantly higher ($P<0.05$) in flower (0.243±0.04 min/mg protein) than in the stem (0.224±0.04 min/mg protein), leaf (0.209±0.03 min/mg protein) and root (0.130±0.04 min/g dry mg protein). Catalase activity was recorded to be significantly higher ($P<0.05$) in flowers (0.009±0.01 units/mg protein) followed by stem (0.008±0.02/units/mg protein), leaf (0.004±0.01/units/mg protein) and root (0.003±0.05 units/mg protein), respectively. Peroxidase activity was found to be significantly higher ($P<0.05$) in flowers (2.836±0.69 units/mg protein) than in stem (1.716±0.04 units/mg protein), leaf (1.583±0.08 units/mg protein) and roots (1.357±0.04 units/mg protein) [Figure 2].

DISCUSSION

Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions.[18] The flower, stem, leaf and root parts are potentially rich sources of natural antioxidants. However, flowers of *Enicostemma littorale* rich in antioxidant enzymes. Many studies indicate a linear relationship between total phenolics and antioxidant activity.[19,20] In another study indicate

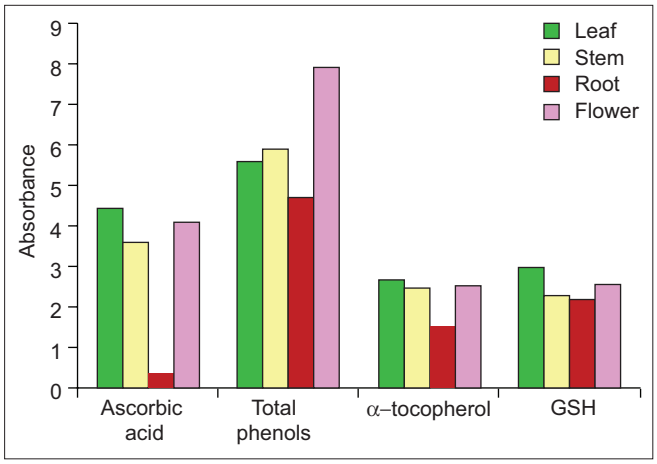

Figure 1: Comparison of non-enzymatic antioxidant activities for parts of *Enicostemma littorale*

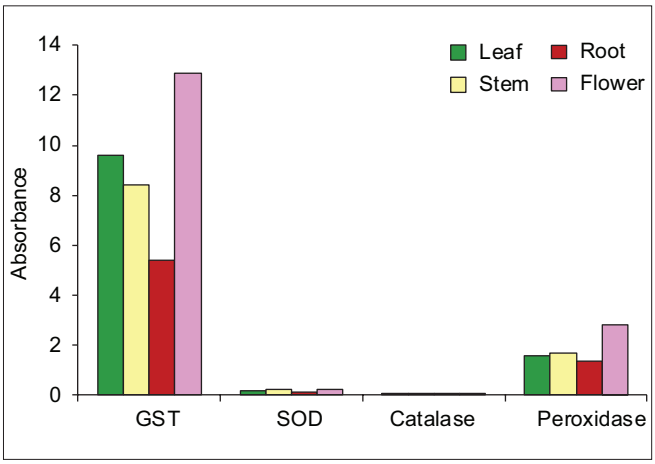

Figure 2: Comparison of enzymatic antioxidant activities for parts of *Enicostemma littorale*

the flowers were potent source of antioxidant activity.[21] The ethanolic extract of dry *Cassia siamea* flowers showed potent antioxidant activity due to the presence of number of polyphenols.[22] In another study reported that, the aqueous and alcoholic extracts of *cassia fistula* leaves showed potential antioxidant activity which may be accounted for by the high phenolic content.[23]

CONCLUSION

In this study, we found that phenolic compounds are major contributors to antioxidant activity, since total phenolics and antioxidant activity showed a good correlation. After this comparative study, our objective will be identification and determination of the amount of individual phenolic compounds responsible for the majority of antioxidant activity the plant.

REFERENCES

1. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
2. Aruoma AM, Butler MJ, Halliwell B. Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives. J Agric Food Chem 1993;41:1880-5.
3. Kirtikar KR, Basu BD. Indian medicinal plants Vol. 3. Dehradun: India: Saujanya Books; 1935.
4. Vasu VT, Modi H, Thaikoottathil JV, Gupta S. Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume aqueous extract in cholesterol fed rats. J Ethnopharmacol 2005;101:277-82.
5. Govindachari TR, Sathe SS, Vishwanathan N. Gentianine, an artifact in *Enicostemma littorale*. Indian J Chem 1966;4:201-2.
6. Vishwakarma SL. Bagul MS. Rajani M. Goyal RK. A sensitive HPTLC method for estimation of swertiamarin in *Enicostemma littorale* blume, *Swetia chitara* (Wall) Clarke, and in formulations containing *E.littorare*. J Planar Chromatogr 2004;17:128-31.
7. Omaye ST, Turnbull JD Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol 1979;62:3-11.
8. Malick CP, Singh MB. Plant Enzymology and histo enzymology. New Delhi: Kalyani Publishers; 1980. p. 286.
9. Backer H, Frank O, De Angelalls B, Feingold S. Plasma tocopherol in man at various times after ingesting free or ocetylaned tocopherol. Nutr Rep Int 1980:21;531-6.
10. Griffith OW, Meister A. Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (*S-n*-butyl homocysteine sulfoximine). J Biol Chem 1979;254:7558-60.
11. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;246:7130-9.
12. Lowry OH. Rosembrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:267-75.
13. Hwang SY, Lin HW, Chern RH, Lo HF, Li L. Reduced susceptibility to water logging together with highlight stress is related to increase in superoxide dismutase and catalase activity in sweet potato. Plant Growth Regul 1999;27:167-72.
14. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
15. Beauchamp C, Fridovich I. Superoxide dismutase: Improve assays and an essay applicable to acetylamide gels. Anal Biochem 1971;44:276-87.
16. Chandlee JM, Scandalios JG. Analysis of variance affecting the catalase development programme in Maize scutellum. Theor Appl Genet 1984;69:71-7.
17. Reddy KP, Subhani SM, Khan PA, Kumar KB. Effect of light and benzyl adenine on dark-treated growing rice leaves, II changes in peroxidase activity. Plant Cell Physiol 1995;24:987-99.
18. Halliwell B, Aruoma OI. DNA damage by oxygen derived species. Its mechanism and measurement in mammalian systems. FEBS Lett 1991;281:9-19.
19. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem 2006;97:654-60.
20. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem 2003;81:321-6.
21. Nagavani V, Raghava Rao T. Evaluation of Antioxidant Potential and Identification of Polyphenols by RP-HPLC in *Michelia champaca* Flowers. Adv Biol Res 2010;4:159-68.
22. Kaur G. Alam MS, Jabbar Z, Javed K, Athar M. Evaluation of antioxidant activity of *Cassia siamea* flowers. J Ethnopharmacol 2006;108:340-8.
23. Siddhurajua P, Mohanb PS, Beckera K. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chemistry 2002;79:61–7.

How to cite this article: Abirami P, Gomathinayagam M, Panneerselvam R. Antioxidant activity of the medicinal plant *Enicostemma littorale* Blume. Int J Green Pharm 2011;5:342-5.

Source of Support: This work is supported by Department of Science and Technology (DST), **Conflict of Interest:** None declared.