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ANTIOXIDANT EFFECT OF *MAJORANA HORTENSIS* LEAVES

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ABSTRACT
Free radicals can be generated in the biological systems in the form of reactive oxygen species which are harmful and these are removed by the antioxidant system in the body. Antioxidants protect us from free radicals that cause tissue damage, neurodegenerative diseases and cancer. Plants are a rich source of antioxidants. In the present study, *Majorana hortensis*, commonly called majoram was chosen as the candidate plant to determine the antioxidant potential. The enzymic activity and non-enzymic levels of the fresh leaves was determined proving the plant to be a potent source of antioxidants.
KEYWORDS: antioxidants, free radicals, reactive oxygen species, oxidative damage.

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INTRODUCTION

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. Current life style is causing the overproduction of free radicals and reactive oxygen species in our body and decreasing physiological antioxidant capacity[1]. Oxidative stress is an imbalance between the factors that exert oxidative stress and those that possess potential antioxidants. Oxidative damage is the consequence of excess oxidative stress, inadequate antioxidant potential or the combination of both[2]. There are several enzymic and non-enzymic antioxidants. However, when the level of free radicals exceeds the ability of the antioxidant system, lipid peroxidation and DNA and protein damage occurs, which results in aging and various diseases, including inflammation, cancer, Parkinson's disease, cardiovascular diseases, multiple sclerosis and lupus[3]. Naturally occurring dietary antioxidants found in medicinal plants can serve as alternatives to chemically designed anticancer agents[4].

Sweet marjoram (*Majorana hortensis* Moench.) is a perennial herb native to Cyprus and eastern Mediterranean countries[5]. The aerial parts of the plants are used for isolation of oil, which has a lot of uses in flavour, perfumery and pharmaceutical industry. In food industry, it is mainly used as a spice in sausages, but its use in baked goods, processed vegetables, condiments, soups, snack foods and gravies are also reported[6].

MATERIALS AND METHODS

Plant Material: The plant was grown in pots after collecting the sapling from Tamil Nadu Agricultural University, Coimbatore and was identified by Botanical Survey of India as *Majorana hortensis* Moench. (voucher number BSI/SC/5/23/08-09/Tech.)

Sample Preparation: An aqueous extract of the fresh leaves 1g/10 ml was prepared and used for all the assays. Both enzymic and non-enzymic antioxidant components were analysed in the fresh leaves of *Majorana hortensis*.

Enzymic Antioxidants: The enzymic antioxidants analysed in the leaves were superoxide dismutase, catalase, peroxidase, glutathione S-transferase and glutathione reductase. Activity of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich (1972)[7]. Catalase (CAT) activity was assayed spectrophotometrically following the method of Luck (1974)[8]. Assay of Peroxidase (POD) as proposed by Reddy *et al.* (1995)[9] was adopted for evaluating the activity of peroxidase. The method of Habig *et al.* (1974)[10] was employed for the assessment of glutathione S-transferase (GST). Glutathione reductase (GR) activity was determined by the method proposed by David and Richard (1983)[11].

Non-Enzymic Antioxidants: The non-enzymic antioxidants analysed in *Majorana hortensis* leaves were

ascorbic acid, tocopherol, total carotenoids, lycopene, reduced glutathione, total phenols and chlorophyll. Estimation of ascorbic acid content in the leaves was carried out by the method of Roe and Keuther (1943)[12]. The level of tocopherol was determined spectrophotometrically by the method of Rosenberg (1992)[13]. Estimation of total carotenoids and lycopene was done as described by Zakaria *et al*. (1979)[14]. The method proposed by Moron *et al*. (1979)[15] was used for the estimation of reduced glutathione. Total phenols were assayed by the method proposed by Mallick and Singh (1980)[16]. The chlorophyll content of the leaves was determined using the procedure described by Witham *et al*. (1971)[17].

RESULTS AND DISCUSSION

From the enzymic antioxidant activities presented in Table 1, it can be deduced that the fresh leaves of *Majorana hortensis* are an excellent source of antioxidants. Table 2 depicts good levels of non-enzymic antioxidants levels in the above leaves in all the assays performed. Medicinal plants are considered to be an important source of antioxidant compounds and the therapeutic benefit of many medicinal plants is often attributed to their antioxidant properties (Hasan *et al*., 2007)[18]. The antioxidant potential of *Clitoria ternatea* L. and *Eclipta prostrata* L. was analysed by Bhaskar *et al*. (2009)[19] by assessing enzymic and non-enzymic antioxidants. Plants having vitamins, flavonoids and polyphenols have been reported to possess remarkable antioxidant activity (Gupta and Sharma, 2006)[20]. Quantitative analysis of the total phenolic content of the seaweeds indicated that *Gelidella acerosa* and *Haligra* species have high phenolic contents, which correlated with their respective antioxidant activity (Devi *et al*., 2008)[21]. Kundu *et al*. (2008)[22] demonstrated that the methanol-aqueous fraction of *Cajanus cajan* leaf extract could prevent the chronically treated alcohol induced rat liver damage by augmenting the antioxidant enzyme activities. In the present study, *Majorana hortensis* leaves also proved to be very good sources of several well-known antioxidants, supporting the hypothesis that the leaves can be exploited for the preparation of medicinal aids to combat oxidative stress-induced diseases and disorders.

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REFERENCES

1. Orrell RW, Lane RJ, Ross M. A systematic review of antioxidant treatment for amyotrophic lateral sclerosis/motor neuron disease. Amyotroph Lateral Sclera 2008;14:1-16.
2. Chow CK, Chang SJ. Value or Usefulness of the food frequency questionnaire for the assessment of dietary total antioxidant capacity. J Nutr 2007;137:1496-1497.
3. Lee BB, Cha MR, Kim SY, Park E, Park, HR, Lee SC. Antioxidative and Anticancer Activity of Extracts of Cherry Blossoms (*Prunus serrulata* var. *spontanea*). Plant Foods Hum Nutr 2008; 62:79-84.
4. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A, Bora U. Indian medicinal herbs as sources of antioxidants. Food Res Int 2008;41:1-15.
5. Verma RS. Aroma profile of Majorana hortensis as influenced by harvesting heights in northern India. Chem Bull 2010;55(69):9-11.
6. Burdock GA. Fenaroli's handbook of flavour ingredients: Natural flavours. 3rd ed. Boca Raton: CRC Press; 1990. p. 202.
7. Misra HP, Fridovich I. The role of superoxide anion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1980;247:3170-3175.
8. Luck H. In: methods in enzymatic analysis. 2nd ed. New York: Bergmeyer
9. Academic Press;1974. p. 805.
10. Reddy KP, Subhani SM, Khan PA, Kumar KB. Effect of light and benzyl adenine and dark- treated graving rice (*Oryza sativa*) leaves - changes in peroxidases activity. Plant Cell Physiol 1995; 26:987- 994.
11. Habig WH, Pabst MJ, Jakoby WB. Glutathione transferase: A first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249:7130-7139.
12. David H, Richard JS. In: Methods of enzymatic analysis, Bergmeyer J, Grab M, editors. 1st ed. Beach Floride:Verlag Chemie Weinheim Deer Field; 1983. p. 358.
13. Roe JH, Keuther CA. The determination of ascorbic acid in whole blood and urine through 2,4-dinitro phenyl hydrazine derivative of dehydro ascorbic acid. J Biol Chem 1943;147: 399-407.
14. Rosenberg HR. Chemistry and physiology of the vitamins. New York: Interscience Publishers; 1992. p. 452-453.
15. Zakaria H, Simpson K, Brown PR, Krotulovic A. Use of reversed phase HPLC analysis for the determination of provitamin A carotenes in tomatoes. J Chrom 1979;176:109-117.
16. Moron MS, Depierre JN, Mannervik VC. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochem Biophys Acta 1979;582:67-68.
17. Mallick CP, Singh MB. Plant enzymology and plant histoenzymology. New Delhi: Kalyani Publishers; 1980. p. 286.
18. Witham FH, Blaydes BF, Derlin RM. Experiments in plant physiology. New York:Van Nostrand; 1971. p. 245.
19. Hasan P, Yasa N, Ghanbari SV, Mohammadirad A, Dehghan G, Abdollahi M. *In vitro* antioxidant potential of *Teucrium Rolium* as compared to α-tocopherol. Acta Pharm 2007;57:123-129.
20. Bhaskar DR, Ravi CK, Madhavi Y, Koteswara PR, Raghava TR. Evaluation of antioxidant potential of *Clitoria ternata* L. and *Eclipta prostrata* L. Indian J Biochem Biophys 2009;46:247-252.
21. Gupta VK, Sharma SK. Plants as natural antioxidants. Natural Product Radiance 2006; 5(4): 326-334.
22. Devi PK, Suganthy N, Kesika P, Pandian SK. Bioprotective properties of sea weeds: *In vitro* evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in

relation to polyphenolic content. BMC Complement Altern Med 2008;8:8-38.
23. Kundu R, Dasgupta S, Biswas A, Bhattacharya A, Pal BC, Bandyopadhyay D, Bhattacharya S, Bhattacharya S. *Cajanus cajan* Linn. (Leguminosae) prevents alcohol-induced rat liver damage and augments cytoprotective function. J Ethnopharmacol 2008;118:440-447.

TABLE 1: Enzymic antioxidant activity of *Majorana hortensis* leaves

ENZYMES	ACTIVITY
SOD (U^{*}/ g leaf)	43.33 ± 0.86
CAT (U^{+}/ g leaf)	103.72 ± 3.58
POD ($U^{\$}$/ g leaf)	19.40 ± 0.02
GST ($U^{@}$/ g leaf)	0.16 ± 0.04
GR ($U^{\#}$/g leaf)	2.50 ± 0.39

The values are mean ± S.D. of triplicates
*1 Unit of enzyme is the amount that causes 50% reduction in NBT oxidation
+1 Unit = Amount of enzyme required to decrease the absorbance at 240nm by 0.05 units
$1 Unit = Changes in absorbance at 430 nm / minutes
@1 Unit = µmol of NADPH oxidized / minutes
1 Unit = nmoles of CDNB conjugated / minutes

TABLE 2: Non-enzymic antioxidant levels of *Majorana hortensis* leaves

PARAMETER	LEVELS
Ascorbic acid (mg/g leaf)	1.70 ± 0.01
Tocopherol (µg/g leaf)	3.59 ± 0.25
Total carotenoids (mg/g leaf)	24.47 ± 0.39
Lycopene (mg/g leaf)	4.26 ± 0.04
GSH (nmoles/ g leaf)	256.19 ± 15.11
Total phenols (mg/ g leaf)	11.81 ± 0.14
Chlorophyll (mg/g leaf)	3.96 ± 0.22

The values are means ± S.D. of triplicates

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