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Research Article

ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACT OF *MENTHA PIPERITA L.*

Valsala P*, Deepika E, Santhy KS

Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, India
**Corresponding author: valsalap001@gmail.com*

ABSTRACT

The accumulation of free radicals in the body leads to many diseases as a result of oxidative stress. Currently, lot of research works have been undertaken to prevent the oxidative stress and also to nullify the effect of free radicals using antioxidants which are essentially from the plant origin rather than the synthetic ones. Peppermint (*Mentha piperita L.*) belongs to the family lamiaceae was used in this study to estimate the amount of enzymatic and non-enzymatic antioxidants present in the methanol extract of the leaves. Here the enzymatic antioxidants such as superoxide dismutase, catalase, peroxidase and the enzymatic antioxidants such as ascorbic acid, α-tocopherol and total phenols were assessed. In the present study the methanol extract of Peppermint (*Mentha piperita L.*) showed a strong amount of antioxidants in the leaves. The leaf extract possesses enzymatic antioxidants like Super oxide dismutase10.05±0.5 U/g, catalase 35.07±0.04 U/g, peroxidase 0.04±0.02 U/g and non-enzymatic antioxidants such as Ascorbic acid 0.99±0.09 (mg/g), α-tocopherol 0.62±0.06 (µg/g), Total Phenols 273.52±6.12 (mg/g). Thus, our findings suggest that peppermint (*Mentha piperita L.*) has the potential to scavenge the free radicals and prevent from the oxidative stress related diseases which pave the way for peppermint to serve as a good phytotherapeutical agent against many diseases and disorders.

Keywords: Peppermint (*Mentha piperita L.*), antioxidants, oxidative stress, free radicals.

1. INTRODUCTION

Peppermint (*Mentha piperita L.*) is an antisepticcross variety of mint (*Mentha spicata*) and water mint (*Mentha aquatic*) belong to the family Lamiaceae. This was initially cultivated within the Mediterranean areas and commercialized in England throughout the late seventeenth century. The utilization of mint oil in mint-flavoured products, fragrances and pharmaceuticals lead to an upsurge in peppermint production over the past few decades [1]. Consequently, the peppermint leaves infusion stands as a widespread drink for its refreshing flavour and peculiar aroma. The Food and Agricultural Organization (FAO/global organization, [2] states that the world production of peppermint in 2010 was about 81,241.00 tones. Africa was the highest producer (71,880.00 tonnes), representing ca. eighty-nine of total production, followed by South America (7100.00 tonnes), North America (2980.1 tonnes), Europe (2231.00 tonnes) and Asia (30.00 tonnes). The therapeutic effects of pepper mint are attributed to the presence of high level of antioxidants. A list of physiological actions has been stated according to the *in vitro*, *in vivo* and human studies which includes, antitumor, anti-microbial, antiallergenic and immunomodulating actions [3, 4]. As an example, flavouring has been found to be a harmless and effective treatment for active petulant bowel syndrome [5, 6]. Though the direct role of aerobic stress in ageing and etiology of persistent diseases remain unproved, stress responses seem to link to ageing, chronic diseases, aerobic stress, and therefore, the free-radical theory of ageing (FRTA) [7, 8]. The FRTA predicts that antioxidants would possibly limit free radical stress to biological structures by detoxifying them and thereby preventing the aerobic stress-related human disorders [9]. Owing to, the in-direct scientific proof, there has been an upsurge of interest within the inhibitor potential of herbal plants. Despite efforts to reveal the presence of chemical compounds related to the peppermint inhibitor property, no comprehensive review has been dedicated. Hence, the current research work aims to deal with this vital issue by performing *in vitro* estimation of enzymatic and non-enzymatic antioxidants in pepper mint.

2. MATERIAL AND METHODS

2.1. Plant collection and sample preparation

Mentha piperita L. (Peppermint) leaves were collected from the areas of Coimbatore. The leaves were washed completely and allowed to dry for 5-7 days at room temperature. The dried-out leaves were ground to powder and stored in screw cap bottles until further analysis.

2.2. Preparation of the extract

A 10gm of sample was dissolved in 100 ml of methanol. They were intermittently shaken with an electric shaker. It was then filtered and further concentrated by evaporation.

2.3. Antioxidant activity

The antioxidant level of *Mentha piperita* L. (Peppermint) leaves were estimated by analyzing various enzymic and non-enzymic parameters.

2.3.1. Enzymatic antioxidant assays

2.3.1.1. Estimation of superoxide dismutase activity [10]

About 300 µl of each reaction mixture which included phosphate buffer (pH 7.8; 50 mM), Methionine (45 mM), 5.3mM Riboflavin, 84mM Nitro Blue Tetrozolium (NBT), and 20 mM potassium cyanide was prepared. This was assorted to 300µl of the sample and subjected to incubation (25°C) which was exposed for 10 min to 15 W fluorescent lamps. The absorbance of the color formed was measured at 600 nm and the reduction of NBT was monitored. The evaluation was done in the absence of enzyme giving 50% inhibition of the reduction of NBT.

2.3.1.2. Estimation of catalase activity [11]

A volume of 3.0ml H_2O_2-phosphate buffer was pipetted into a cuvette, and the enzyme extract (0.01 - 0.04) was added to it. Mixture was then mixed thoroughly. Time taken for the decrease in absorbance by 0.05 units was recorded. This value was used for calculations. If it exceeded more than 60 seconds, repeated the measurement with more concentrated solution of the sample.

2.3.1.3. Estimation of peroxidase activity [12]

The assay mixture containing 3.0ml of pyrogallol solution and 0.1ml of enzyme extract were taken in a cuvette. The spectrophotometer was adjusted to read zero at 430 nm followed by the addition of 0.5ml of 1% H_2O_2 and mixed. The change in absorbance was recorded every 30 seconds for 3 minutes. One unit of peroxidase activity refers to the variation in absorbance perminute at 430 nm.

2.3.2. Non-Enzymic antioxidant assays

2.3.2.1. Estimation of ascorbic acid [13]

The assay volumes were made up 2.0ml with 4% TCA. 0.2 to 1.0ml of the working standard solution containing 20-100 µg of ascorbate respectively were pipetted out into cleandry test tube, the volume of which were also made up to 2.0ml with 4% TCA. Added 0.5mlof DNPH reagent to all the test tubes, followed by 2 drops of 10% thiourea solution. The sample was incubated at 37°C for 3 hours. The osazones formed were dissolved in 2.5ml of 85% sulphuric acid, in cold, drop bydrop, with no appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of H_2SO_4. The tubes were incubated for30 min at room temperature, and the absorbance was read spectrophotometrically at 540nm. The quantity of ascorbic acid in the sample was calculated using the standard graph.

2.3.2.2. Estimation of phenols [14]

The diluted extract was taken and the volume was made up to 3.0ml with distilled water. Then 0.5ml of Folin Ciocalteau reagent and 2.0ml of 20% superoxide dismutaseium carbonate solution were added. After mixing the tubes thoroughly, the blue solution obtained was warmed for a minute, cooled andthe recorded the absorbance at 650 nm against a reagent blank. Standard curve was prepared in an electronic calculator set to the linear regression mode using known concentrations of catechol solution (0.2-1.0ml) corresponding to 2.0-10µg. Total phenol content in the sample was calculated using a standard curve and the values are expressed as mg phenols/g tissue

2.3.2.3. Estimation of tocopherol [15]

A volume of 1.5ml of the plant extract, standard and water (test, standard and blank) was pipetted out into three centrifuge tubes. To all the tubes, 1.5ml each of ethanol and xylene were added, mixed well and centrifuged. After centrifugation, the xylene layer was transferred into another tube, without disturbing the ethanol or protein layer. To 1.0ml of xylene layer, 1.0ml of 2,2'-dipyridyl reagent was added and mixed. This reaction mixture was taken in the spectrophotometric cuvettes and the extinctions of the test and

the standard was read against the blank at 460 nm. Then, in turn, beginning with the blank, 0.33ml of $FeCl_3$ solution was added, mixed well and after exactly 15 minutes, the test and the standard were read against the blank at 520 nm. The results are expressed as μg tocopherol/g of sample.

Tocopherol (μg) = $A_{520} \times A_{460} / A_{520}$ x 0.29 x 15

3. RESULTS AND DISCUSSION

Naturally occurring antioxidants in plant cells are the potent scavengers of free radicals and protect the human body from several diseases caused due to free radical reactions. Antioxidant nutrients have the capability in scavenging free radicals which neutralize them prior to any damage to body cells. Plants scavenge the reactive oxygen species (free radicals) by producing enzymes like superoxide dismutase, glutathione peroxidase, glutathione reductase, ascorbate oxidase and glucose 6-phospate dehydrogenase. They are the principal ROS-scavenging systems in plants [16]. The enzymic antioxidants analyzed in the present investigation were catalase, superoxide dismutase and peroxidase. These are the preventive antioxidants and first line of defense against ROS.

The activities of enzymatic antioxidants namely catalase, super oxide dismutase and peroxidases were analyzed. Table 1 depicts the levels of enzymatic antioxidants. Super oxide dismutase and catalase in *peppermint*were found to be 10.05±0.5 and 35.07±0.04 U/g tissue respectively. In this study the peroxidase level was found to be 0.04±0.02 U/g tissues. Among the four enzymes, catalase showed highest level of35.07±0.04 U/g tissue.

Table 1: Enzymatic antioxidant levels in the methanol extract of peppermint leaves

Parameter (U/g)	Level
Super oxide dismutase[a]	10.05±0.5
Catalase[b]	35.07±0.04
Peroxidase[c]	0.04 ± 0.02

[a] *1Unit = Amount of enzyme that gives 50% reduction in NBT oxidation.*

[b] *1Unit = Amount of enzyme required to decrease the absorbance at 240 nm by 0.5 units*

[B] *1Unit = Change of absorbance/minute at 430 nm.*

Catalase is the most important and efficient antioxidant known today [17]. It is a tetrameric enzyme catalyzes the reduction of hydrogen peroxides into water and oxygen and thus protects tissues from highly reactive hydroxyl radicals. Among the four enzymes studied, catalase showed highest activity which coincides very well with the activity of superoxide dismutase indicating that H_2O_2 formed by superoxide dismutase is effectively removed by the catalase. Similar studies were conducted by Rao and Suresh (2013) [18] to elucidate the antioxidant efficacies of aqueous and methanolic extracts of leaves of *Cassia tora* and *Cassia sophera*. *C. tora* showed higher concentration of superoxide dismutase and Vitamin C, while *C. sophera* exhibited more of catalase, guaicol peroxidase, reduced glutathione and protein content when compared to *C. tora*. Raghavan and Krishnakumari [19] examined the effect of ethanolic extract of *Terminalia arjuna* stem bark in alloxan induced diabetic rats and its lipid peroxidation, enzymatic and non-enzymatic activity in the liver and kidney tissues. The extract caused a significant increase in superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase, reduced glutathione, vitamin A, vitamin C and vitamin E in liver and kidney of alloxan induced diabetic rats. Super oxide dismuatse is another important defense enzyme, which catalyzes the dismutation of superoxide radicals to oxygen and hydrogen peroxide and acts as a substrate for subsequent catalase action. As an enzyme, superoxide dismutase has particular value that can help to protect against cell destruction processes. Superoxide dismutase are reported widely in plant sources and are currently used in the treatment of anti-oxidative and anti-inflammatory diseases [20]. It is believed that superoxide anion, arising either through metabolic processes or following oxygen "activation" by physical irradiation, is considered the "primary" ROS or can further interact with other molecules to generate "secondary" ROS, either directly or prevalently through enzyme- or metal-catalyzed processes [21]. Peroxidases are heme containing enzymes which are able to oxidize organic and inorganic compounds using hydrogen peroxide as co-substrate. It seems that their activity plays a major role in peroxide removal in peppermint.

The non-enzymic antioxidants analyzed in the present investigation were ascorbic acid (Vitamin C), α-tocopherol (Vitamin E) and phenols. The level of non enzymic antioxidants is shown in Table 2. Of the three enzymes analyzed, the level of phenol was found to be maximum (273.52±6.12). The level of α-tocopherol

was found to be 0.62±0.06μg/g and Ascorbic acid was found to be 0.99±0.09mg/g.

Table 2: Non-enzymatic antioxidant levels in the methanol extract of peppermint leaves

Parameter (U/g)	Level
Ascorbic acid (mg/g)	0.99±0.09
α-tocopherol(μg/g)	0.62±0.06
Total Phenols (mg/g)	273.52 ± 6.12

These are the second line of defense against free radicals. When a free radical was destroyed by an antioxidant, this antioxidant itself becomes oxidized. Therefore, the antioxidant resources must be constantly restored in the body [22]. Here comes the role of non-enzymic or dietary antioxidants. Ascorbic acid is a water soluble vitamin, provides intracellular and extracellular aqueous-phase antioxidant capacity primarily by scavenging oxygen free radicals. Vitamin C works synergistically with vitamin E to quench free radicals and also regenerates the reduced form of vitamin E. Vitamin E is a fat-soluble vitamin with high antioxidant potency. As it is fat-soluble, α-tocopherol safeguards cell membranes from damage by free radicals. Its antioxidant function mainly resides in the protection against lipid peroxidation. Vitamin E triggers apoptosis of cancer cells and inhibits free radical formations [23]. Our results indicated that vitamin C level was comparatively high in rhizome whereas the level of vitamin E was found to be lowest. Similar results were reported by Karthikeyan and Rani [20] who evaluated the enzymatic and non-enzymatic antioxidants in selected piper species.

Phenols are the secondary compounds that occur naturally in plants with better physiological potential that enhance the healthy human body. Polyphenols seems to be an important factor for dietary antioxidant activity. Phenolic compounds like flavonoids protect human body from harmful free radicals, whose formation is associated with the normal natural metabolism in aerobic cells. Natural polyphenols are capable of destroying free radicals, chelate metal catalysts and activate the enzymes responsible for antioxidant capacity [24]. The quantity of phenolics is very high in the infused extracts which supply 88% to 95% of total polyphenols at the foremost infusion [25]. By the way, a studied was reported that peppermint infusion has approximately 750 mg/L of phenolic compounds were the intake of peppermint tea daily about 500 ml could add up to 375 mg of phenolics [26]. Phenolic acids generally tend to possess antiinflammatory and antioxidant properties. Phenolic compounds are generally recognized to perform as free radical scavengers by their reducing properties, which act as hydrogen or electron donors, singlet oxygen quenchers and metal chelators [27]. Rosmarinic acid is the most important phenolic compounds in infusions from the family Lamiaceae (e.g., *Rosmariunm officinalis, M. piperita*) [27].

4. CONCLUSION

The study reports that the peppermint (*Mentha piperita L.*) is the reservoir of enzymatic and non-enzymatic antioxidants. This could be developed as a good therapeutic agent that minimizes the oxidative stress thereby preventing the human body from many degenerative diseases and disorders like cancer, diabetes, obesity and cardiac problems. The present study demonstrated that the peppermint possess the strong antioxidant defense mechanism which might be utilized as a source of natural antioxidant and an alternative to enzyme such as superoxide dismutase, catalase and peroxidase. Essential oil or leaf infusions of peppermints have a pack of the naturally occurring antioxidants. Usage of mint leaves is usual in food and its consumption could be credited to the oxidation-reduction stability in human cells, due to the presence of the entire phytochemicals such as vitamins, phenolics and terpenic antioxidants in the leaves. This mixture of antioxidants may act powerfully on the overallcomplex of oxidative stress, which might tend to have a great impact on ageing and other related illnesses.

5. ACKNOWLEDGEMENTS

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Conflict of interest

The authors declare no conflict of interest

6. REFERENCES

1. Lawrence BM. *CRC Press*, Boca Raton, FL, 2006.
2. FAO/UN (Food and Agriculture Organization ofthe United Nations). FAOSTAT: data on peppermint yield and production by continent, 2010
3. Lv J, Huang H, Yua L, Whent M, Niu Y, Shi H, etal. *Food Chemistry*, 2012; **132:**1442-1450.
4. Alam MS, Roy PK, Miah AR, Mollick SH, KhanMR, Mahmud MC, et al. *Medical Journal*, 2013; **22:**27-30.
5. Barja G. *Prog in Mol Bio and Translat Sci.,* 2014; **127:**1- 27.

6. Canistro D, Boccia C, Falconi R, Bonamassa B, Valgimigli, L, Vivarelli F, et al. *J of Gerontol Ser: A Biomed Sci and Med Sci.,*2015; **70(8):**936-943.
7. Khanna R, MacDonald JK, Levesque BG. *J of Clini Gastroentero.*, 2014; **48:**505-512.
8. Park SH, Ozden O, Jiang H, Cha YI, Pennington JD, Aykin-Burns N. *Int J of Mole Sci.*, 2011; **12(9):**6226-6239.
9. Misra HP and Fridovich I. *J.Biol. Chem.*, 1972; **247(3):**3170-3175.
10. Luck H. Biogneyer: *Academic Press*. 1974: 85- 88
11. Reddy KP, Subhani SM, Khan, PA, Kumar B. *Plant Cell. Physiol.*, 1995; **26(4):**987-944.
12. Roe JH, Keuther CE. *J. Biol.Chem.*, 1953; **147(5):**399-405.
13. Malick CP, Singh MB. *Kalyani Publishers*, New Delhi, 1980; 286-289.
14. Rosenberg H.R. *Interscience Publishers Inc.*, 1992; 452-543.
15. Liu ZJ, Zhang XL, Bai JG, Suo BX, Xu, PL, Wang L. *Sci Hortic.*, 2009; **121:**138-143.
16. Rahman K. *Clinical Interventions in Aging*, 2007; **2(2):** 219-236.
17. Rao S, Suresh C. Brit *Biotec J.,* 2013; **3(3):**377-389.
18. Raghavan B,Krishnakumari S. *Ind J of Phys and Pharm*, 2006; **50(2):**133-142.
19. Karthikeyan J, Rani P. *Indian J. Exp. Biol.*, 2003; **41:**135-140.
20. Valko M, Morris H, Cronin MD. *Curr. Med. Chem.*, 2005; **12:**1161-1208.
21. Young I, Woodside J. *J. Clin. Pathol.*, 2001; **54:**176-186.
22. White E, Shannon JS, Patterson RE. *CancEpidemiol.Biomark Prev.*, 1997; **6:**769-774.
23. Amic D, Davidovic D, Beslo D, Trinajstic N. *Croatia Chemica Acta.*, 2003; **76(1):**55-61.
24. Fecka I, Turek S. *J of Agri and Food Chem.*, 2007; **55:**10908-10917.
25. Duband F, Carnat AP, Carnat A, Petitjean-FrevtetC, Clair C, Lamaison JL. *Ann PharmaceutiqFrançai.*, 1992; **50(3):**146-155.
26. Oh J, Jo H, Cho AR, Kim SJ, Han *J. Food Cont.*, 2013; **31:**403-409.
27. Kapp K, Hakala AE, Anne Orav A, PohjalaL, Vuorela P, Pussa T, et al. *FoodRes Intern.*, 2013; **53(2):**758-766.