Research Article

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In Vitro Antioxidant activity of *Mentha pulegium* from Saudi Arabia

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Mentha pulegium which is locally know as Al-Medina mint, is used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor. The objectives of this study were to examine the antioxidant activity and the total phenol and flavonoids contents of *Mentha pulegium* methanol extract. The *In vitro* antioxidant activity of Mentha pulegium methanol extract was evaluated using various methods, such as ferric-ferricyanide reducing assay and Cupric reducing antioxidant capacity assay. Total phenolic and flavonoid content of *Mentha pulegium* methanol extract were also determined. The obtained results showed that *Mentha pulegium* methanol extract have a very high phenol and flavonoid content (157.99 ± 12.3 mg GAE/g DW and 16.96 ± 1.48 mg RTE/g DW respectively). The methanol extract showed a significant reducing activity nearly equivalent to vitamin C. The results presented here indicate that *Mentha pulegium* cultivated in Saudi Arabia possess strong antioxidant activity.

Key words: Al-Medina mint, antioxidant activity, phenolics, flavonoid, reducing power.

Reactive oxygen species (ROS) produced either normally as a by-product of normal metabolism or due to external factors, such as radiation and drugs, cause damages to biomolecules and thereby compromise cell viability (Halliwell and Gutteridge, 2004).

Increased production of ROS is implicated in the pathogenesis of many diseases including; cancer, cardiovascular diseases, Down's syndrome, Friedreich's ataxia, rheumatoid arthritis, autoimmune diseases and acquired immunodeficiency syndrome. Oxidative damage is also emerging as an important factor in mutagenesis, tumorigenesis, ageing and age-related disorders such as Parkinson's and Alzheimer's diseases. (Halliwell, 1997).

Epidemiological studies have confirmed that intake of exogenous antioxidants is effective in preventing or suppressing many diseases, therefore, there is a growing interest in natural phenolic antioxidants present in medicinal and dietary plants that might help preventing or decreasing oxidative damages without exerting harmful side effects (Silva, et al., 2005).

Furthermore, there are growing interests in using natural products for preventive and therapeutic medicine due to their minimal side effects (Jo, et al., 2008).

The genus Mentha includes 25-30 species that grow worldwide (Dorman et al. 2003). In Saudi Arabia the most common species of *Mentha is Mentha pulegium* which is locally know as Al-Medina mint, and is used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor (Moreno et al. 2002). In addition, Mentha spp. has been used as a folk remedy for treatment of nausea, bronchitis, anorexia, ulcerative colitis, and liver diseases (Iscan et al. 2002). Several studies have examined the antioxidant activity of Mentha species growing in various region of the world (Hajlaoui. et al.,

2009; Yumrutas and Saygideger, 2012). But there are no detailed reports on antioxidant activity of Mentha pulegium growing in Saudi Arabia.

The objectives of this study were to examine the antioxidant activity and the total phenol and flavonoids contents of Mentha pulegium methanolic extract growing in Saudi Arabia using various methods, such as ferric-ferricyanide reducing assay and Cupric reducing antioxidant capacity assay.

MATERIALS AND METHODS

All chemicals used are of analytical grade and were obtained from Riedel and Scharlau, Germany and LobaChemi, India. *Mentha Pulegium* was purchased from local market in Al-Madina city, Saudi Arabia.

Estimation of total phenols

Total phenol content of the methanol extract of *Mentha Pulegium* was estimated by using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). The method based on reaction between Phenolic compounds with phosphomolybdic acid in Folin reagent to produce a blue-colored complex in alkaline medium, which can be estimated spectrophotometrically at 765nm.

The original procedure was modified to use with total volume of 1.5 ml instead of 5 ml used in the original method. Briefly 0.15mL of the sample dissolved in MeOH was incubated with 0.75 mL of 10% FC reagent and 0.6 ml of 7.5% Na_2CO_3 for 30 min at room temperature. The absorbance of the color developed was measured at 765 nm against blank. The total phenolic content was expressed as gallic acid equivalents (GAE) using a standard curve generated with gallic acid as standard.

Total flavonoid content

Total flavonoid content was determined by a colorimetric method described previously (Eberhardt et al. 2000). The original procedure was modified to use with total volume of 1.5 ml instead of 2.5 ml used in the original method. Briefly, 0.2 ml of the extract was diluted with 0.78 ml of distilled water. Then 60 µl of a 5% NaNO2 solution was added to the mixture. After 5 min, 60 µl of a 10% $AlCl_3$ solution were added, and the mixture was allowed to stand for another 5 min at room temperature. a 400 µl of 1 M NaOH was added. The solution was well mixed, and the absorbance was measured immediately against the prepared blank at 510 nm using a spectrophotometer in comparison with the standards prepared similarly with rutin. The results are expressed as milligrams of rutin equivalents/mg (mg RE/mg).

Ferricyanide reducing assay

The reducing ability of the methanol extract of *Mentha Pulegium* was determined according to the method of Oyaizu et al. (1986). The method based on formation of blue colored complex due to reduction of ferric (Fe^{3+}) form to ferrous (Fe^{2+}) form by antioxidant.

The original procedure was modified to use with total volume of 1.7 ml instead of 8 ml used in the original method. Briefly Different concentrations of sample in 0.2 ml of MeOH were mixed with 0.5 ml of Phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of 1% potassium ferricyanide. The mixture was incubated for 20 minutes at 50° C. At the end of the incubation, 0.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 5000 rpm for 10 minutes. 0.5 ml of the upper layer was mixed with 0.5 ml of distilled water and 0.1 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm. A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as a positive control.

Cupric reducing antioxidant capacity (CURAC assay)

The cupric ion reducing antioxidant capacity of *Mentha pulegium* methanol extract was determined according to the method of Apak et al. (2008). The original procedure was modified to use with total volume of 1.5 ml instead of 4.1 ml used in the original method. Briefly, according to the protocol 0.036 mL of sample extract was mixed with 0.366 mL each of Cu II acetate solution (10mM), neocuproine alcoholic solution (7.5 mM), and NH4Ac(1M, pH 7.0) buffer solution and 0.366 mL of water to make the final volume 1.5 ml. After 30 min, the absorbance was recorded at 450 nm against the reagent blank. Standard curve was prepared using different concentration of gallic acid (500, 250, 125, 62.5 µg/ml). The results were expressed as µg GAE/mg

Statistical analysis

Experimental analyses were performed in duplicate. Data were recorded as mean ± standard deviation and analyzed by

GraphPad prism version 5.

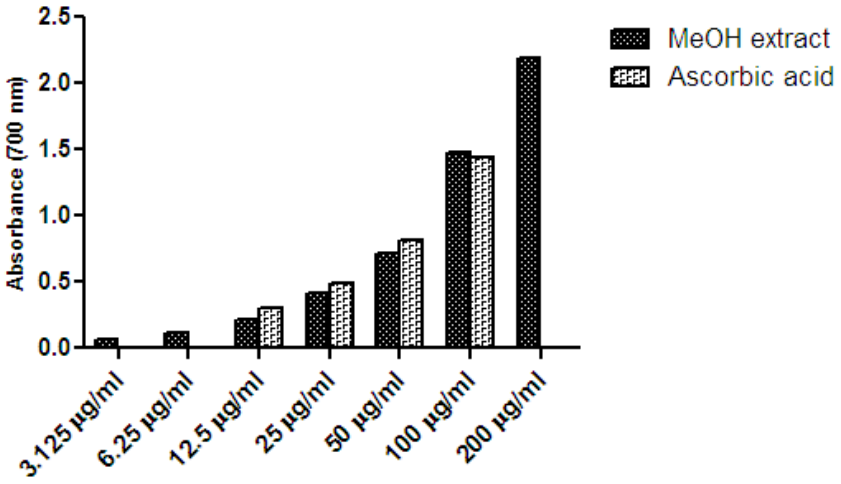

Figure 1: Fe^{3+} reducing power of *Mentha pulegium* methanol extract. Values are mean ± S.D. of three experiments

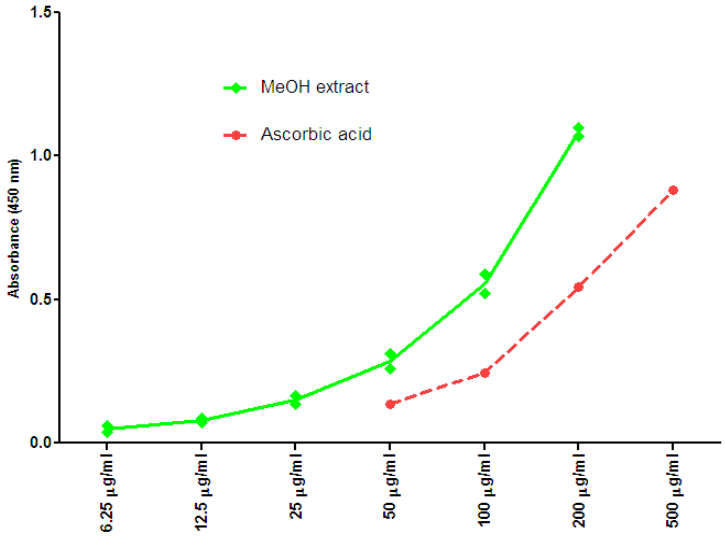

Figure 2: Cupric reducing antioxidant capacity of *Mentha pulegium* methanol extract. Values are mean ± S.D. of three experiments

Table 1: Total phenol & flavonoid contents of *Mentha pulegium* methanol extract. Values are mean ± S.D. of three experiments.

	Total phenol (mg GAE/g dry weight)	Total flavonoid (mg RTE/g dry weight)
Mentha pulegium MeOH extract	157.99 ± 12.3	16.95 ±1.48

RESULTS AND DISCUSSION

Determination of total phenol & flavonoid content

Phenolic and flavonoid compounds have been shown to be responsible for the antioxidant activity of plant materials (Rice-Evans et al., 1996). Therefore, the amount of total phenol & flavonoid in the methanol extract of *Mentha pulegium* were determined by using Folin-Ciocalteu and $AlCl_3$ reagent

respectively (Table 1).The methanol extract of *Mentha pulegium* was shown to be very rich in phenol content (157.99 ± 12.3 mg GAE/g. The flavonoids content in the methanol extract of *Mentha pulegium* was found as 16.96 ± 1.48. The contents of polyphenols or flavonoids were overall more significant than those described by Karray-Bouraoui et al. (2010) who reported that the phenolics contents of Tunisian *M. pulegium* showed wide ranges of 20.1–56.6 mg GAE/ g DW, and 12.9–52.1 mg CE /g DW for total phenols and flavonoids, respectively. Although other study (Yumrutas and Saygideger, 2012) showed high level of phenol and flavonoids of Turkish *Mentha pulegium* methanol extract (206.58 ± 4.54 mg GAE/ g DW and 46.54 ± 1.05 mg CE /g DW respectively).

Reducing power assays

The reducing power of a compound serve as a significant indicator of its potential antioxidant activity, because its associated with their ability to donate electron to free radical species, reducing them into more stable and un-reactive form (Serbetci and Gulcin, 2010). Therefore the reducing power of essential oil samples were investigated using 2 methods; Ferricyanide reducing and cupric reducing antioxidant capacity (CURAC) assays

Ferricyanide reducing power

The ability of essential oil samples to reduce Fe^{3+} to Fe^{2+} was determined and compared with that ascorbic acid (Figure 1)**.** All used concentration of *Mentha pulegium* methanol extract showed some degree of electron donating capacity to reduce Fe^{3+} to Fe^{2+} in a dose dependent manner. According to the obtained result the methanol extract of *Mentha pulegium* have a significant reducing power which was comparable to that of ascorbic acid.

Cupric reducing antioxidant capacity (CURAC) assay

Further, the reducing ability of *Mentha pulegium* methanol extract was confirmed by measuring its ability to reduce Cu^{2+} to Cu^{1+} ions and result was compared with ascorbic acid as standard. All used concentration of *Mentha pulegium* methanol extract showed some degree of electron donating capacity to reduce Cu^{2+} to Cu^{1+} in a dose dependent manner. According to the obtained result the methanol extract of *Mentha pulegium* have a significant reducing power which was even more than that of ascorbic acid (Figure 2).

Conclusion

The obtained results showed that *Mentha pulegium* methanol extract cultivated in Saudi Arabia have a very high phenol and flavonoid content and possess strong antioxidant activity.

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