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Antioxidant Activity of Dietary Plants: Peppermint

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Abstract: Aqueous and methanolic extracts of *Mentha piperita* were analyzed for their phenolic and flavonoid compounds content which were 10.93, 23.43 and 5.850, 17.38% for aqueous and methanolic extracts respectively. The reducing power of the peppermint of aqueous extract and methanolic were also determined, it was find that the reducing power was enhanced by increasing concentration of samples. It was 80.27% for aqueous extract at 10mg/ml concentration while the reducing power of methanolic extract was 88.00% at the same concentration. Results showed that the differences between two methods extraction may be due to the extract solvent and what compounds can be gain by it. Aqueous extract showed low chelating capacities in comparative with EDTA compound, the absorbance were 0.09 and 0.28, respectively at 10mg/ml while it was higher than methanolic extract with absorbance 0.04.

Key words: Antioxidant activity, peppermint, phenolic compounds

INTRODUCTION

Much attention has been focused on the activity of the natural antioxidants present in fruits and vegetables, because potentially these components may reduce the level of oxidative stress (Thomas, 1994). Several epidemiological studies suggest that a high intake of food rich in natural antioxidants, increase the antioxidant capacity of the plasma and reduces the risk of some. The properties are attributed to a variety of constituent, including vitamins, minerals, fibers and numerous phytochemicals, including flavonoids (Wang *et al*., 1996). Probably the most important natural phenolic compounds are flavonoids because of their broad spectrum of chemical and biological activities (Vundac *et al*., 2007). Peppermint is currently one of the most economically important aromatic and medicinal crops produced in the USA (Eccles, 1994). Peppermint leaves and oil are used for folk medicine, as flavoring agents and in cosmetic and pharmaceutical products (Foster, 1996). It is one of the world's oldest medicinal herbs and is used in both Eastern and Western traditions (Eccles, 1994). *Mentha peperita* follow Labiatae family, It's named (Naanaa bustani) or (Naanaa fulfuly) in some areas, peppermint is a small herb with square stems usually reddish-purple and smooth; the flowers are purple, terminal cluster in the end of the stem. Leaves used in food as spices to give acceptable taste and help to support the appetite (Qutub and Fawzi Taha, 1981). Peppermint is taken internally as a tea, tincture, oil or extract and applied externally as a rub or liniment. Herbalists consider peppermint an astringent, antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, mild bitter, analgesic, anticatarrhal, antimicrobial, rubefacient, stimulant and emmenagogue (Hofiman, 1996; Bore, 1996). It has potentially active chemical constituents like: volatile oils, monoterpenes, caffeic acids, flavonoids and tannins (Fleming, 1998; Robbers and Tyler VE. Tyler's 1999). So they have biological activities: antimicrobial, antiviral and antifungal besides potential clinical benefits (Paula, 2000). In the present study, we focused on evaluating the antioxidant activity from natural source, in crude aqueous and methanolic extracts of peppermint leaves.

MATERIALS AND METHODS

The peppermint leaves were locally obtained, cleaned and ground. 20 g of ground material was extracted by 250 ml distilled water or ethanol 95% at boiling point, under reflux for 1 h. The extractive was filtered and evaporated at 50°C to the compete dryness.

Determination of total phenolic compounds: A Folin-ciocalteu calorimetric method was used as described by (Biglari *et al*., 2008). To a 0.5 ml of (1mg/ml) extract a 2.5 ml of a ten-fold diluted Folin-ciocalteu reagent and 2 ml of 7.5%. Sodium carbonate solution were added before the reaction allowed standing for 30 min at room temperature. The absorbance was recorded at 760 nm by using Pye unicum spectrophotometer. The total phenolic compounds were determined according to gallic acid standard curve (Fig. 1).

Determination of flavonoids: The total flavonoids in aqueous and methanolic extracts were determined according to (Zhisben *et al*., 1999). 1 ml extract solution (1mg/ml) was placed in 10 ml volumetric flask. 5 ml of distilled water and 0.3 ml of 5% $NaNO_2$ solution were added. After 5 min 0.6 ml of 10% $AlCl_3$ was added. 2 ml of 1M NaOH solution was added after another 5 min and the volume was made up to 10 ml with distilled water.

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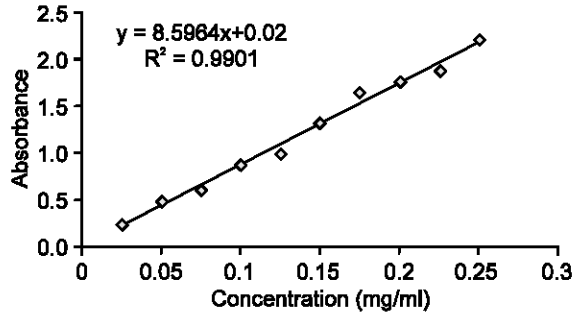

Fig. 1: Concentration-response curve for gallic acid at 760 nm

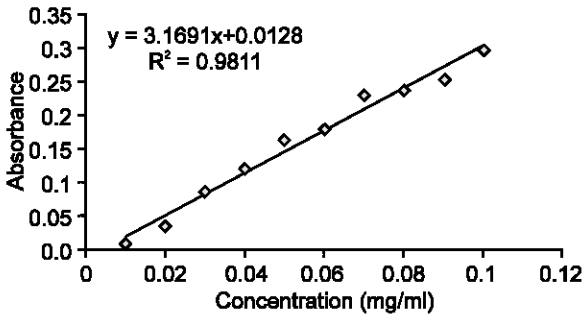

Fig. 2: Concentration-response curve for catechin at 510 nm

The mixture was mixed thoroughly and the absorbance was measured at 510 nm. The flavonoid compounds were determined according to catechin standard curve (Fig. 2).

The assay of antioxidant activity

The reducing power: The reducing power was estimated as described by Chou *et al.* (2009). 1 ml extract of (2-10mg/ml) was mixed with 2.5 ml of 1% potassium ferric cyanide and 2.5ml of 0.2M (pH, 6.6) of sodium phosphate buffer and incubated at 50°C for 20 min. To stop the reaction, 2.5 ml of 1% Trichloroacetic acid (TCA) was added to the mixture and centrifuge for 10 min at 3000 rpm. 0.5 ml of the supernatant was mixed with 1ml of 1% ferric chloride and stand for 10 min. The absorbance was measured at 700 nm. 0.02% of BHT used as reference.

The chelating ability: Chelating ability was determined according to (Su *et al.*, 2008) with some modification. 1 ml of (2-10mg/ml) extract was mixed with 0.2 ml ferric chloride of 2mM and 0.2 ml 8-Hydroxyquinoline (5mM). After 10min at room temperature, the absorbance was determined at 562 nm. The EDTA-Na_2 was used as reference.

RESULTS AND DISCUSSION

Aqueous and methanolic extracts of *Mentha piperita* were analyzed for their phytoconstituents. The

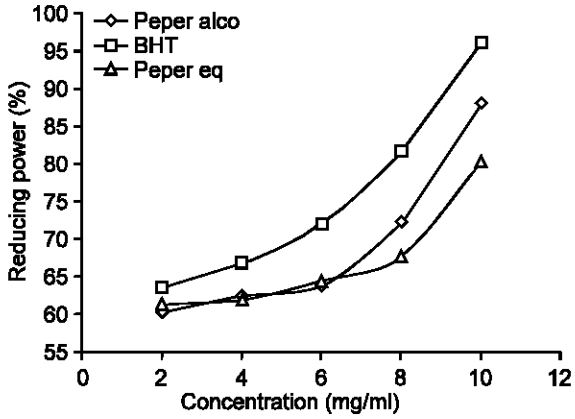

Fig. 3: The Reducing power of methanolic and aqueous extracts of peppermint.

Table 1: Flavonoids content in peppermint extracts

Extraction	Type of plant	PC (%)	FC (%)
Aqueous	peppermint	10.937	5.850
Methanolic		23.4378	17.385

PC: Phenolic content, FC: Flavonoids content

quantitative estimation of the content of total phenolics and flavonoids were 10.93, 23.43 and 5.850%, 17.38% for aqueous and methanolic extracts respectively (Table 1). The phenolic content was found to be much higher in methanolic extract than in aqueous extract, may be correlated to the solvent of extraction. *Mentha piperita* is rich in total phenols (Gardiner, 2000). Reducing power indicates compounds that are electron donors which can act as primary and secondary antioxidants (Yen and Cheu, 1995). The reducing power of the peppermint of aqueous extract were in the following order 61.29, 62.02, 64.48, 67.81 and 80.27% at 2-10mg/ml, respectively while the reducing power of methanolic were 60.26, 62.46, 63.96, 72.32 and 88.00% at the same concentrations (Fig. 3). From these results we can find that the reducing power was enhanced by increasing concentration of samples. Higher reducing power might be attributed to higher amounts of total phenolic and flavonoid and the reducing power of a compound may reflect its antioxidant potential (Lee *et al.*, 2007). The phenolic compounds have been recognized as antioxidant agents which act as free radical oxidation terminators (Peckman and London, 1980), the reducing properties are generally associated with the presence of reductions (Shimada *et al.*, 1992). The results showed that the differences between two methods extraction may be due to the extract solvent and what compounds can be gain by it. Water was selected as the extraction solvent since it is commonly used in the food industry in a variety of ways. Water and methanolic extracts were subjected to screening for their possible antioxidant activity, the decrease in the absorption is taken as a measure of the chelating capacities of the extract.

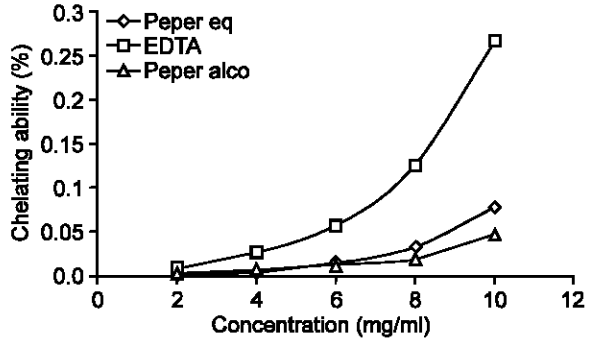

Fig. 4: Chelating ability of methanolic and aqueous extracts of peppermint.

Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial, antithrombotic, antimutagenic and anti-carcinogenic activities (Benavente-Garcia and Castillo, 2008). Aqueous extract showed low chelating capacities in comparative with EDTA compound, the absorbance were 0.09 and 0.28, respectively at 10mg/ml while it was higher than methanolic extract with absorbance 0.04 (Fig. 4). These results agreed with many researchers, they found that the chelating ability for ethanolic extraction of dates was between 59-70% at 4, 6, 8 and 10mg/ml concentration (Balasundram *et al*., 2005) while others found that the chelating ability for aqueous extract of dates was 67% at 10mg/ml concentration and it was 82-87% for DMSO dates extracts (Rohman *et al*., 201; Ozsoy *et al*., 2008). On the other hand chelating ability for alcoholic extract was 47.19% for pomegranates, 57.23% for fig, 48.58% for black Grape at 5mg/ml concentration (Al-Hilfi, 2009). Extract of (Deglet noor) dates had antioxidants which due to wide range of phenolic compounds in it (Alidin *et al*., 2003). It was found that binding compounds form bounds with metals and it acts as secondary antioxidants (Kumaran and Karunakarna, 2006).

Conclusion: Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals by preventing or delaying damage of cells and tissues. The antioxidant activities of vegetables varied largely. In both systems tested of their mechanisms of antioxidant actions; chelating metal ions to prevent the generation of free radicals and reducing power percentage. The antioxidant activity correlated with active compounds phytochemicals such as phenols, tannins, flavonoids ….etc. In the present study, the methanolic extracts of peppermint leaves exhibited strong reducing power ability, in addition the extract contains amount of phenols and flavonoids higher than the aqueous extracts. The results imply that peppermint may be used as an antioxidant, leading to the possibility of developing natural antioxidant material.

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