

Variation of Antioxidant Activity of *Melissa officinalis* Leaves Extracts During the Different Stages of Plant Growth

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ABSTRACT

Plants rich source of phenolic compounds (phenolic acids, flavonoids and tannins) are the most important natural antioxidants. The *Melissa officinalis* has many valuable properties including antioxidant, anti-bacterial, analgesic, and relaxing. The purpose of this study was to determine change of antioxidant activity of plant leaves in various stage and the best harvest time of *M.officinalis* leaves for maximum antioxidant activity. Lemon balm leaves in three stages Different (vegetative growth stage, flowering stage and the stage after of flowering) collected and was dried. The antioxidant capacity of the plant extract done DPPH. The highest total phenolic and total flavonoids in leaves of plants and DPPH in flowering stage was high. So it seems the best time to plant, harvest leaves for the operation of its antioxidant property is in August.

Key words: Antioxidant activity, *Melissa officinalis*, FRAP assay, Radical scavenging, flavonoids, Total Phenols.

INTRODUCTION

The high levels of free radicals in biological systems are able to oxidize biomolecules, which leads to tissue damage, cell death, or a various of diseases such as cancer, heart disease, inflammation and high sensitivity¹⁹. Antioxidant compounds can deactivate and scavenge the free radicals. Antioxidants can inhibit the effect of oxidants by donating hydrogen atom or chelating metals (Gülcin *et al.*, 2003; Parkash *et al.*, 2007). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as additives in food to prevent oxidation of lipids. Plants rich in compounds antioxidant can protect cells from oxidative stress²². In this family of plants containing large amounts of anti Lamiaceae are antioxidants. *Melissa officinalis* belongs to family of lamiaceae, a large group of medicinal plants. Balm (*Melissa officinalis* L.) is an aromatic (lemony) perennial

herb, up to about 1 m high, growing in the Mediterranean region, Western Asia, South-Western Siberia and North Africa. Parts mostly used are dried leaves, often having flowering tops^{20, 3}. *Melissa officinalis* (lemon balm) is an important herb among the indigenous medicinal plants of genus *Melissa* (Zargari, 1990). It is a well known herb used to give fragrance to different food and beverage products. This too, has been used as traditional medicine to cure nervousness, headaches and gastrointestinal disorders³.

Essential oils and extracts of this plant have been reported to have, antiviral (Schnitzler *et al.*, 2008), antimicrobial and antioxidant properties¹⁷.

Plant phenolic compounds that are natural antioxidants from various plant sources. Phenolic compounds can act as a reducer (free radicals) and oxygen unique and chelating of metals⁵.

Studies have shown that received antioxidants through food plays effective roles in maintaining and improving health. For example, coronary artery disease and certain cancers is reversed associated with the consumption of food polyphenols. These studies have led to the attention to natural antioxidant molecules resources. Wach Research and co-workers in 2005 showed that the receiving source of phenols and flavonoids in various region world are dependent on kind of nutritious people. For example, in countries such as Japan, the supplier of green tea consumption providing composition object of needed while this components are supplied in Western countries with consuming onion and apple and in Eastern countries with consuming vegetable and fermenting food^{32,7}. In our country, especially in the north of region, the high consumption of vegetables, plus fiber, vitamins and salts, can be a good source of natural antioxidants. The leaves *M. officinalis* have been used in folk medicine especially in Iran and Turkey, for the treatment of some diseases²⁷. Also, the leaves of *M. officinalis* are often used as herbal teas. *M. officinalis* contains some phenolic and flavonoid compounds such as rosmarinic acid²⁰. Flavonoids have a antioxidant properties because have Phenolic structure²⁴. This plant is due to compounds such as Rosmarinic acid has antioxidant properties. Phenolic content in plants, have some antioxidant properties¹³.

Shahsavari and co-workers expressed the presence of phenolic compounds carvacrol and thymol as the cause of the high antioxidant activity of essential oils of *Thymus vulgaris* L²⁹.

More than 300 kinds of herbal extract containing *Melissa officinalis* and it is produced in Europe (Schultze *et al.*, 1989). Some activities may be related to the phenolic component of lemon balm, including Rosmarinic acid, tannins and flavonoids.

The leaves of the medicinal properties of plants be related to contact antioxidant, the purpose of this study is changes in antioxidant activity of extracts of *Melissa officinalis* leaves in various stages of development and determination good time harvesting leaves of *M. officinalis* for having high antioxidant activity to be used best for the pharmaceutical industry. For this purpose, the study

the antioxidant properties of extracts of leaves, total phenolic and flavonoid content was also measured.

MATERIAL AND METHODS

Preparation of plant samples lemon balm have collected in three different times of growth plant (vegetative growth, flowering stage and after flowering) respectively, in 10 June, 19 August and 23 September 2011 from the regional Tonekabone in province of Mazandaran. Then for extraction leaves of *M. officinalis* was used Soxhlet apparatus. For this work 20 to 30 grams of dried leaves plant and Methanol solvent for extraction was used. The time of extraction was about 7 hours. Extract was transferred to a glass jar and lid in the oven 50 degrees Celsius for 24 hours was maintained until the remaining solvent to evaporate. The extract was ready for tests.

Antioxidant activity

Membrane lipids rich in unsaturated fatty acids that make them susceptible to oxidative processes in the company. Inhabitation of peroxidation fats may be due to free radical scavenging of antioxidants activity. Hydroxyl radicals led to reducing the hydrogen atoms of the lipid membrane. The atoms of led to peroxidation of fats.

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay

The DPPH radical is one of a few stable organic nitrogen radicals, which bears a deep purple color. It is commercially available. This assay is based on the measurement of the reducing ability of antioxidants toward DPPH. The ability can be evaluated by electron spin resonance (EPR) or by measuring the decrease of its absorbance. The widely used decoloration assay was first reported by Brand-Williams and co-workers¹². Antioxidant assay are based on measurement of the loss of DPPH color at 515 nm after reaction with test compounds, and reaction is monitored by spectrometer.

The aliquots of plant extract (20-40-60-80-100 μ L) were mixed with a methanol solution of DPPH (1 mM, 600 μ L) and brought to 6 mL with methanol.

After incubation in the dark at room temperature for 15 min, absorbance of the solutions was measured at 517 nm. A DPPH blank sample (containing 5.4 mL of methanol and 600 μ L of DPPH solution) was prepared and measured daily.

Ascorbic acid was used as the standard in concentration (10, 5, 2.5, 1.25 and 0.625 micrograms in ml water) and with the method of the above was added to solution of DPPH. Three replicates was considered for each concentration. control extracts were used to remove color of extracts. Finally, the absorbance of solution after 30 minutes stay in the dark, was measured by spectrophotometer UV in the wavelength of 517 nm. The percentage scavenging of the DPPH radical (RSA) was calculated by the following formula:

$$\text{RSA (\%)} = [1 - (S - SB) / C] \times 100$$

In this formula S and SB are, respectively, absorbance of the sample (DPPH + extract) and the control (methanol + extract) and standard (DPPH + methanol). Then obtain of percentage scavenging of the DPPH radical (RSA), IC_{50} values of extract and ascorbic acid were determined. The IC_{50} value, which represents the concentration of extract that gives rise to a 50% reduction in DPPH absorbance, was determined by linear regression analysis. The quantity of mixture of antioxidants needed to reduce by 50% the initial DPPH concentration was evaluated. This characteristic parameter is called IC_{50} (Chung *et al.*, 2006).

Total antioxidant capacity

Total antioxidant capacity was measured by reduction of ferric (Fe^{+3}) form to the ferrous (Fe^{+2}) form because of their reductive capabilities (Chung *et al.*, 2006). Reducing Iron III is often revived as an indicator of activity electrons can be used as an important mechanism is action phenolic antioxidant substances²¹.

Ferric reducing antioxidant power (FRAP) assay is a simple and reliable colorimetric method and commonly used for measuring the antioxidant capacity (Benzie & Strain, 1996). Briefly The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$

and 25 mL of 300 mM acetate buffer, pH 3.6 were prepared. The mixture was shaken vigorously for 30 sec and warmed at 37°C Then Freshly prepared FRAP reagent 3ml mixed with 150 μ L of extract and the reaction mixtures were later vortexed. The absorbance at 593nm was evaluated after 15 min at room temperature and the data were translated into FRAP values (μ M), using a methanolic solution of Fe(II) in the range of 200–2000 μ M ($FeSO_4 \cdot 7H_2O$) for calibration FRAP was used as control of reactive. The $FeSO_4 \cdot 7H_2O$ aqueous solution with concentrations (2000, 1000, 500, 250 and 125 micromoles per liter) for Calibration curves were plotted. FRAP value was expressed in micromolar iron sulfate on gram plant extracts. ascorbic acid as the standard concentrations (200, 100, 50, 25 and 5 / 12 mg ml) was used (Benzie and Strain, 1996).

Total flavonoid content

3000, 2000 and 1000 micrograms ml extract concentration were prepared in methanol. The standard routine concentration of in methanol was prepared in the following: 50, 100, 150, 200 and 250 micrograms in ml.

The blank solution was made up with distilled water

50 cc of 5% solution sodium nitrite ($NaNO_2$) in distilled water was prepared. 50 cc of 10% solution aluminum chloride ($AlCl_3$) in distilled water was prepared. 100 cc of 1 M (NaOH) was prepared in distilled water.

1 cc of each extract concentration, the standard and blank was mixed with 4cc distilled water. 0.3 cc of 5% sodium nitrite was added and mixed well. After 5 minutes, then 0.3 cc of 10% aluminum chloride was added and the mixed well. After 6 minutes, 2 cc of NAOH was added and mixed with distilled water (3.4 ml). Then we have a volume of 10 cc. The absorbance at 510 nm was measured. All measurements were performed in triplicate. All tests was performed in one day (Chung *et al.*, 2002, Jamshidi *et al.*, 1389).

Total phenolic content

The concentration of the extract, 3000, 2000 and 1000 micrograms per milliliter in methanol were prepared. The vary concentrations standards

of gallic acid was made up with distilled water the following: 50, 100, 150, 200 and 250 micrograms per ml was used for madding blank the solution. Then 300 cc of solution (Na_2CO_3), sodium carbonate 7% were prepared in distilled water. 1 cc of each concentration extract, the standard and blank is added in balloon 25 cc containing of distilled water 9 cc. 1 cc Folin-Ciocalteu's reagen was added and mixed well. After 5 minutes, then 10 cc of sodium carbonate 7% of prepared was to added and mixed well. After 90 minutes the absorbance was measured at 750 nm. Phenolic content was calculated based on the standard curve of gallic acid. Results were presented as the mean of duplicate analyses and expressed as milligrams of gallic acid equivalents per gram dry weight (mg of GAE per g dw).

Gallic acid was used as the standard and the results were expressed as mg/L gallic acid equivalents (GAE). All measurements were performed in triplicate. All tests was performed in one day (Hinneburg *et al.*, 2006; Chung *et al.*, 2006).

RESULTS

The results showed that the flavonoid content in plants *M.officinalis* based on routine mg g extract was obtained. Vegetative growth stage (before flowering) 92.42 mg Rutin / g, in the flowering stage of 118.43 mg / Rutin / g and the flowering of 90.44 mg Rutin / g respectively (Fig. 1).

The total phenolics were determined according to the Folin-Ciocalteu method. Total phenolic content was showed in the three stages of the harvest before flowering, flowering and after flowering, respectively 23.15 mg GAE / g, 38.77 mg GAE / g and 20.16 mgGAE / g (Fig. 2).

Total phenolic was increased in flowering stage of plant and reducing in after flowering stage. The total absorption rate increased with increasing extract concentration thus total phenol will increase

In this study, the antioxidant power of methanolic extract of lemon balm leaves by the

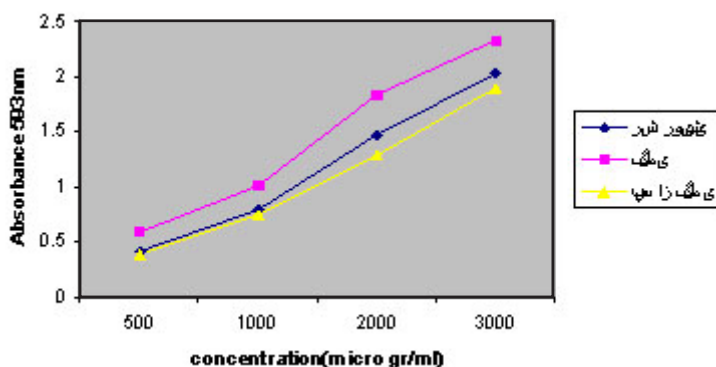


Fig. 1: Absorbance of extracts for determining total flavonoid in vary stage of *M.officinalis*

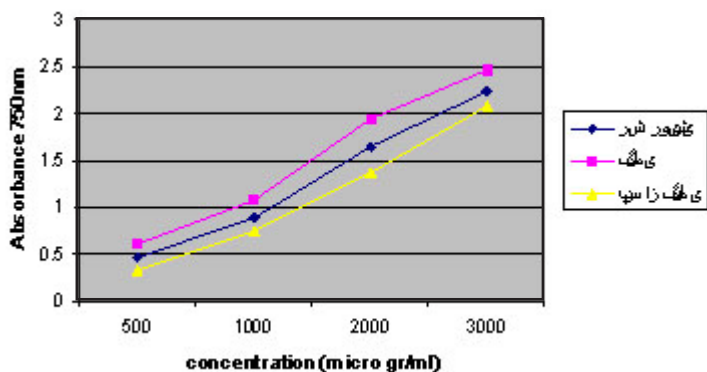


Fig. 2: Absorbance of extracts for determining total phenolic in vary stage of *M.officinalis*

DPPH and FRAP assay was obtained. The results of the three phases was obtained actively (vegetative growth, flowering and maturity stages of seed) 1.2922, 1.0591 and 1.0481 g/l (FRAP assay and in DPPPH assay was 10.95, 13.36 and 13.28 ml/l.

Using excel and SPSS (ANOVA) and mean comparisons were performed and all data obtained were statistically significant.

CONCLUSION

DPPH is a stable free radical with the nitrogen atom of hydrogen or electron capture processes by reducing the color from purple to yellow becomes. Compounds that have the capability to perform this action are as an antioxidant (Brand-Williams *et al.*, 1995). In this study the antioxidant activity of plant *Melissa officinalis* at different stages of growth showed that the transition from the vegetative plant, the amount of antioxidant activity in leaves of the plant increases. The phenolic compounds in leaves are connected with antioxidant properties, it is likely that the amount of the compound or its active forms of the antioxidant activity in these organs are more or less at the time specified. This is probably the antioxidant compounds in the vegetative leaves are small and gradually to reach the flowering stage is increased. High antioxidant activity in leaves after the flowering period, depending on the appearance of new shoots or storage process has started. Accumulation of secondary products such as French researchers Rosmarinic acid that can operate to trap free radicals have also proved to be important (Lamaison *et al.*, 1991). Other possibilities is about the presence caffeic acid, rosmarinic acid and frolic acid in *Melissa officinalis*¹⁶.

FRAP levels were lower in leaves after of flowering. Antioxidant activity was highest at flowering time, perhaps was due to high levels.

Rosmarinic acid at this time and phenolic OH groups with the effect low.

Phenolic and flavonoid compounds that are present in leaves are associated with antioxidant property

Phenolic compounds reducing after the flowering period can be caused by the breakdown secondary metabolites to simple intermediate metabolites

of initial. Consequently increase the antioxidant activity of the flowering stage to conform to Porbozorgi and co-workers studies on plants of basil (*Ocimum basilicum* L.) (1385).

Total phenols and flavonoids reducing of after flowering plants may be because most of the flavonoid in plants are a natural glycoside² and research on changes in glucose metabolism in root, in various stages of development in various plants including *Symphytum officinalis* showed that the glucide reduction in flowering stage and the vegetative growth of plants is increased. The antioxidant effects of plant essential oils and extracts are mainly due to the presence of hydroxyl groups in their chemical structure (1,8).

Due to the presence of phenolic compounds with antioxidant properties and medicinal plant, isolation, identification and determination the exact composition and these changes in different stages of development seems to be necessary.

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