

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Antioxidant Activity of Gilan *Mentha pulegium* During Growth

Rezvan Shahmohamadi, Reyhaneh Sariri, Mahdi Rasa and Mahmudreza Aghamali
Department of Biology, University of Guilan, Rasht, Iran

Abstract: Antioxidant activity and total phenolic contents of methanolic and hydroalcoholic-acetone extracts of Iranian *Mentha pulegium* in two stages of maturity were investigated. The aim was to investigate the most suitable solvent for extraction of antioxidants and to find the correlation existed between plant growth stage and its antioxidant capacity. *In vitro* antioxidant properties of the extracts were examined by 1, 1 diphenyl-2-picrylhydrazyl (DPPH) and Ferric Reducing/Antioxidant Power (FRAP) methods. Moreover, total phenolic contents were determined by the Folin-Ciocalteu method. On the other hand, the phenolic compounds were analyzed by chromatographic methods, TLC and SPE-reversed phase-HPLC. The results revealed that the antioxidant capacities and total phenol contents of the extracts in flowering season were higher than pre-flowering season. A positive relationship was found between the results obtained from three different assay methods used, i.e. FRAP, DPPH and phenol contents. The TLC chromatogram of the two extracts showed differences in the number of separated compounds of extracts. HPLC results indicated that the fraction collected with washing buffer (pH = 6) had highest antioxidant activity.

Key words: *Mentha pulegium*, DPPH, FRAP, TLC, SPE-reversed phase-HPLC

INTRODUCTION

New evidence derived from literature review have shown that Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are related to the pathogenesis of many neurodegenerative diseases including multiple sclerosis (Van Horsen *et al.*, 2008), Alzheimer's Disease (AD) (Smith *et al.*, 2000), Parkinson's Disease (PD) (Bolton *et al.*, 2000), Mild Cognitive Impairment (MCI) (Guidi *et al.*, 2006), cardiovascular disease and cancer (Kris-Etherton *et al.*, 2002). The imbalance between production and breakdown of these species increases the risk of chronic diseases. The antioxidant defense system inhibits free radical damage in our body. Natural antioxidants in fruits, vegetables and herbs can play a great role in supporting our body's defense system (Ali *et al.*, 2008).

In the recent years, plant-derived antioxidants have received extensive attention from both food preservation industries and pharmaceutical companies (Hai *et al.*, 2012). The phytochemical components including phenolic acids, flavonoids, carotenoids and ascorbic acid are good alternatives for synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) whose side effects on human health have been frequently reported (Ito *et al.*, 1983).

Mentha is an aromatic plant belonging to the Labiatae family. The genus of *Mentha* is comprised of

20 species that spread throughout the world. *Mentha pulegium* is one of the *Mentha* species known as pennyroyal, a native herb of Asia, near East, Europe and North Africa (Chalchat *et al.*, 2000). In the flora of Iran, the *Mentha* genus is represented by 6 species (Mozaffarian 1996). *Mentha pulegium* L. has been traditionally used as antiseptic for treatment of cold, sinusitis, cholera, food poisoning, bronchitis and tuberculosis (Zargari, 1990). In traditional medicine, it has some possible effects as antifatulent, carminative, expectorant, diuretic and antitussive. Its larvicidal, acaricidal and cytotoxic activities have also been investigated (Atmani *et al.*, 2009). Mahboubi and Haghi (2008) have reported cytotoxic effect of the essential oil of *M. pulegium* against different human cell lines (Shirazi *et al.*, 2004). Furthermore, it has been reported that the essential oil and extracts of *M. pulegium* can exerts antimicrobial and antioxidant effects (Mahboubi and Haghi, 2008; Shahmohamadi *et al.*, 2011; Nickavar *et al.*, 2008). Although *M. pulegium* is frequently used in Gilan folk medicine, a scientific research on the biological properties of the plant was not found from this region.

Therefore, the aim of present study was comparing the antioxidant activity of *M. pulegium* by two known antioxidant tests (FRAP and DPPH) as well as total phenol content in two different extracts of it during two stages of maturity. On the other hand, it was planned to determine the correlation between antioxidant properties and

phenolic constituents of the extracts. Finally it was aimed to investigate its phenolic compounds by partial purification of the extracts using chromatographic techniques, including TLC and SPE-reverse phase-HPLC.

MATERIALS AND METHODS

Chemicals and reagents: The 1,1 diphenyl-2-picrylhydrazyl (DPPH), 2,4,6 tripyridyl-s- triazine (TPTZ), sodium acetate, ferric chloride, sodium carbonate, sodium hydroxide, gallic acid, Folin-Ciocalteu's phenol reagent and ferrous sulphate were purchased from Sigma representative in Iran. Chromatographic standard was obtained from Sigma. All solvents, methanol, acetone, acetonitrile and ethyl acetate were of analytical grade and purchased from Merck.

Plant materials: *Mentha pulegium* L. samples were collected during the months of April (pre-flowering stage) and August (flowering stage) 2011 from Saravan. The place is a region located in about 40 km from the Caspian Sea in Guilan Province, North of Iran. The taxonomic identity was confirmed by comparing the specimen with those of known identities collected in the Herbarium of the Department of Biology, University of Guilan. Fresh leaves were transferred to the biochemistry research laboratory, washed, drained and air dried for two weeks in the absence of light at room temperature. The dried samples were weighed, marked and stored in opaque paper bags.

Preparation of the extracts: The samples were extracted by two solvent systems, pure methanol and water-methanol-acetone (50-30-20). Extraction procedure was similar to the method of Wojdylo *et al.* (2007) with slight modification. Briefly, 0.5 g of the dried ground plant samples and 10 mL of solvents were added to test tube and the suspensions were shaken softly. Suspensions were sonicated twice for 15 min and left at room temperature (25°C) for 24 h. The extracts were filtered using Whatman No. 1 paper. The filtrates were centrifuged for 10 min in 1500 g and supernatants were collected and stored in at 4°C.

Estimation of antioxidant activity

General free radical-scavenging ability

DPPH Assay: The free radical-scavenging activity was determined using DPPH° radical described by Brand-Williams *et al.* (1995). This method is based on the reduction of stable DPPH when it accepts hydrogen from an antioxidant compound. Radical scavenging activity of methanol and methanol-acetone-water extracts against stable DPPH° was determined spectrophotometrically. The change in color (from deep-violet to light-yellow) was

measured at 515 nm using a uv/visible light spectrophotometer (Ultrospec 3000 from Pharmacia Biotech). Briefly, 100 µL of various concentrations of each extracts was added to 1500 µL of fresh solution of DPPH° in methanol (6×10^{-5} mM) and mixed. The samples were kept in the dark for 30 min at room temperature and then the decrease in absorption was measured. The absorption of a control containing the same amount of methanol and DPPH° solution was then measured. Each experiment was carried out in triplicate. Radical scavenging activity was calculated using the following relationship:

$$\text{Inhibition (\%)} = \left[\frac{(A_0 - A_1)}{A_0} \right] \times 100$$

where, A_0 -absorption of control, A_1 -absorption of extract solution

Ferric reducing antioxidant power (FRAP) assay: The Antioxidant Activity (AOA) of extracts was determined using the ferric reducing ability of plasma (FRAP) assay as described by Benzie and Strain (1999). The FRAP reagent was prepared by mixing 10 volumes of 300 mmol L⁻¹ acetate buffer, pH 3.6, with 1 volume of 10 mmol L⁻¹ 2,4,6 tripyridyltriazine (TPTZ) in 40 mmol L⁻¹ hydrochloric acid and with 1 volume of 20 mmol L⁻¹ ferric chloride. Freshly prepared FRAP reagent was shaken at 37°C for 10 min, then 3 mL of this reagent was added to each sample solution and mixed thoroughly. The absorbance was read at 595 nm after 30 min. A standard curve was prepared using different concentrations (0.1-1 mmol L⁻¹) of FeSO₄ · 7H₂O (1 mM). The results were corrected for dilution and expressed in mmol FeSO₄ L⁻¹. All solutions were used in the day of preparation. All determinations were performed in triplicate.

Determination of total phenol content: Total Phenolic Content (TPC) in extracts was determined according to the Folin-Ciocalteu procedure described by Gao *et al.* (2000). In short, an aliquot of 100 µL of extracts was mixed with 0.2 mL Folin-ciocalteu phenol reagent and 2 mL of H₂O and incubated at room temperature for 3 min. A 1 mL of 20% sodium carbonate solution was then added and allowed to stand for 1 h at room temperature. The absorbance of the reaction mixture was read at 765 nm using a uv/visible light spectrophotometer (Ultrospec 3000 from Pharmacia Biotech). An aqueous gallic acid solution (100-1000 mg mL⁻¹) was used for calibration. Total phenol content was expressed as milligrammes Gallic Acid Equivalents (GAE) per 1 g of dry weight (dw). All determinations were performed in triplicate.

Polyphenolic analysis by thin-layer chromatography: Thin-layer chromatography was used for initial isolation to detect the active components in plant extracts. About

5 μ L of methanolic and ethanolic extracts were loaded on silica gel plate (10 \times 10 cm, Merck F₂₅₄) and developed in horizontal tanks using a solvent system consisted of ethyl acetone:methanol:water (77:13:10) (Carnat *et al.*, 2005). The separated components were visualized under uv light.

Fractionation of phenolic compounds by SPE procedure:

In order to partially concentrating the extracts, ion exchange chromatography and ethyl silica gel cartridge were used. Briefly, 500 μ L of extract was applied to a column ethyl Silica Gel (100 mg, 1 mL) was from Supelco. The extracts were taken to a column which was previously balanced with washing buffer (buffer phosphate with pH 5). Then the buffer phosphate with different pH values (6, 7, 8.5, 10, 11.5) were used for washing. All fractions were collected and antioxidant activity in each fraction was examined using DPPH reagent.

HPLC-DAD system for analysis of phenolic compounds:

A 20 μ L extract samples were analyzed using an Agilent 1200 Series system (Agilent Technologies) composed of a G1379A degasser, a G1311A quaternary pump and a G1316A column oven set at 30°C, containing a ZORBAX Eclipse plus C18 (250 \times 4.6 mm, 5 μ m) column with a flow rate of 1 mL min⁻¹, coupled to a G1315B Photodiode-Array Detector (PAD) set to scan from 190 to 400 nm. All samples and solvents were filtered through a 0.22 μ m filter (Machery-Nagel membrfilter prorafil) before injection

solvent A methanol: acetonitrile: water (5:5:95) and solvent B the same system in the proportion of (5:95:5). The gradient system was as follows: 5% B (0-6 min), 25-40% B (6-15), 40-60% B (15-16). Detection was carried out at 366 nm. Phenolic compounds were identified by comparing retention times and uv-vis spectra with those of standards. The calibration curve of rosmarinic acid was calculated at 366 nm. The results were expressed as mg rosmarinic acid per 100 g of dry weight (dw) samples.

Statistical analysis: All experiment were repeated at least three times and the data were expressed as Mean \pm SD and two-way ANOVA. Means were compared using Duncan's Multiple Range Test with significant level of 0.05 and curves were drawn using Microsoft Office Excel program.

RESULTS AND DISCUSSION

Estimation of antioxidant activity

DPPH Assay: The stable organic radical, DPPH, has been widely used to investigate the free radical scavenging activity of different plant extracts (Katalinic *et al.*, 2006). The radical scavenging activities of both extracts obtained from *M. pulegium* leaves in pre-flowering and flowering stages are presented in Fig. 1a. It was found that water-methanol-acetone extract in both stages (pre-flowering and flowering) exhibited into HPLC column. The mobile phase was composed of

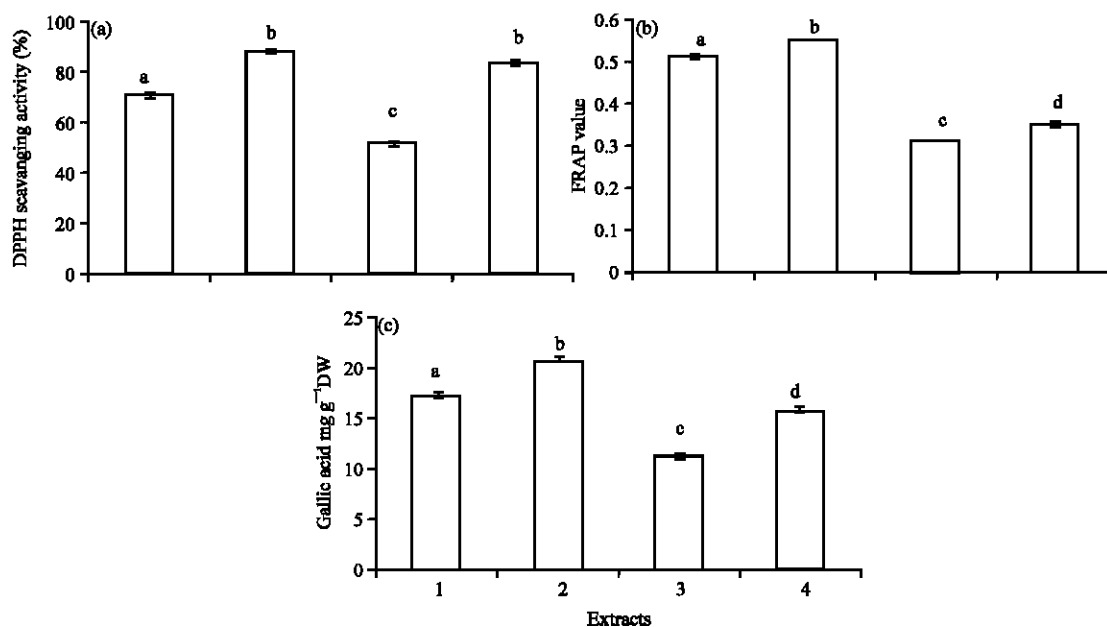


Fig. 1(a-c): (a) DPPH radical scavenging activity, (b) FRAP assay, (c) TP content in the extracts of *M. pulegium* 1; Methanolic extract in flowering stage, 2; Water-methanol-acetone extract in flowering stages, 3; Methanol extract in pre-flowering stage, 4; Water-methanol-acetone extract in pre-flowering stages. Significant differences are shown with different letters (p<0.05)

significantly ($p < 0.05$) highest scavenging activity, 84.03 and 87.9%, respectively. On the other hand, the DPPH scavenging activity by methanolic extracts in pre-flowering and flowering stages were 51.76% and 70.78%, respectively which was weaker than water-methanol-acetone extracts (Ali *et al.*, 2008). It has been reported that polar solvents could extract portions with higher radical scavenging activity compared to non-polar solvents (Tepe *et al.*, 2005). A subsequent study has shown that radical scavenging properties of the water extracts are higher than the methanolic extracts (Kamkar *et al.*, 2010). This characteristic of the extracts can be ascribed to their phenolics, flavonoids and terpenoids contents. Antioxidant activity of water extracts are higher than organic extracts because active components such as flavonoid, terpenoid, polyphenol, carotenoid and sterol, exist in fiber section of plants and are more easily dissolved in water (Rahmat *et al.*, 2003). Our results were supported by some similar investigations, for example, Spigno *et al.* (2007) reported that the solvent used for extraction as well as time and temperature affect antioxidant activity of the extracts. The results obtained from the present study also indicate that there was no statistically significant difference between the antioxidant activities of water-methanol-acetone extracts in both stages.

FRAP assay: In this study, the ability of extracts to reduce Fe^{+3} to Fe^{+2} was determined by FRAP assay. This is a simple method to estimate total antioxidant capacity as FRAP value. As can be seen in Fig. 1b, the highest total antioxidant capacity was significant ($p < 0.05$) in flowering stage in water-methanol-acetone and in methanolic extracts, 0.557 and 0.515 mmol Fe mL^{-1} , respectively. Moreover, the FRAP value was highest in water-methanol-acetone extracts at both stages ($p < 0.05$). The obtained data in pre-flowering and flowering stages were 0.352 and 0.557, respectively. The antioxidant activity of Labiate family has also been evaluated previously by other authors. All plants from this family have shown great capacity in reducing ferric, Fe^{+3} to ferrous ion, Fe^{+2} (Wojdylo *et al.*, 2007). The results obtained by Kumar and Patra (2012), on *M. piperita* have indicated that the FRAP value was poor in the leaves before flowering, it was then considerably increased during the flowering stages and the poorest reported value was after flowering stage. Szollosi has explained that the higher total antioxidant capacity of leaves in flowering period could be due to the great amount of phenolic compounds and reducing power of its OH-groups. He also reported that content of rosmarinic acid was great in leaves of *M. piperita* (Kumar and Patra,

2012). The FRAP value obtained in present study was similar to the results reported by Szollosi. Diversity in antioxidant activity of extracts could be due to the difference in polarity of solvent as well as the ability of solvent to extract antioxidant compounds differently.

Total phenol content (TPC): TPC for both extracts in pre-flowering and flowering period was determined using Folin-Ciocalteu method. The results were expressed as mg gallic acid per gr dry weight of plant sample as shown in Fig. 1c. The TPC determined by Folin-ciocalteu reagent doesn't show the exact amount of phenolic antioxidants, as many non-phenolic compounds such as ascorbic acid, beta-carotene and Cu (I) can also react with this reagent (Sultana *et al.*, 2007). The results showed that water-methanol-acetone extract in flowering stages have the highest total phenol content 20.76, whereas methanolic extracts in pre-flowering stages have the lowest content of total phenol 11.32 mg GAE/g dw. It has been suggested that the members of Labiate family are remarkably rich in polyphenols (Wojdylo *et al.*, 2007; Kosar *et al.*, 2005; Nickavar *et al.*, 2008) have reported higher amounts of total phenol in ethanolic extract of *M. pulegium* than the values we obtained in this study. The difference between the results of different researches may be related to extraction method, polarity of solvent, environmental differences (climate, location and time harvesting of sample) and also the examination methods (Kim and Lee, 2004). The results obtained by the present research showed that more than type and polarity of solvent season of sample collection was effective in amount of polyphenol content and antioxidant activity.

Our findings were in agreement with some previous studies reported by other researchers. For example, Moure *et al.* (1999) reported that it is much better to use more polar solvents for extraction of polyphenols. Matkowski *et al.* (2008) has found that in extracts of Labiate family the polar fractions had greater amount of total phenol in comparison to non-polar fractions. This may be due to the higher concentration of hydroxyl cinamic acid derivatives in polar fractions. On the other hand, many studies have explained that time of harvesting, maturity and age of samples affected the amount of phenolic compounds (Ghasemnezhad *et al.*, 2011; Wang *et al.*, 2007) have investigated the influence of the maturation of peanuts on the total phenol content of the hulls and the antioxidant properties of their methanolic extracts. Their results indicated that amounts of polyphenols increased during maturity. The findings of present study is in agreement with previous researches and has shown that the time of harvesting of samples affected the amount of the total phenol content and antioxidant ability of extracts.

Relationship between total phenol and antioxidant activity:

While some authors have reported a correlation between polyphenol contents and antioxidant properties (Wojdylo *et al.*, 2007; Katalinic *et al.*, 2006; Atmani *et al.*, 2009), others have shown poor or no correlation between phenolic composition and antioxidant properties of some plants (Capecka *et al.*, 2005; Ismail *et al.*, 2004). Therefore, the antioxidant activity observed could be due to the presence of other compounds such as ascorbic acid, tocopherol and some pigments (Li *et al.*, 2005). Our investigation indicated a relatively direct correlation between the total phenol content and radical scavenging activity of extracts ($R^2 = 0.81$) on one hand and between the total phenol content and the total antioxidant capacity ($R^2 = 0.75$) on the other. Wojdylo *et al.* (2007) have reported high correlation between antioxidant activity as measured DPPH and FRAP as well as TPC ($R^2 = 0.83, 0.91$) in Labiatae family. These surveys propose that phenolic compounds with reducing properties associated with their OH-groups, are responsible for the reasonably high antioxidant capability of *M. pulegium* as medicinal plant.

Chromatographic analysis: The phenolic compounds in ethanolic and methanolic extracts of both stages (pre-flowering and flowering) of *M. pulegium* were analyzed by TLC and viewed under UV 366 nm (Fig. 2). The revelation with UV indicated that ethanolic and methanolic extracts have different bonds. The TLC chromatogram of the two extracts showed differences in

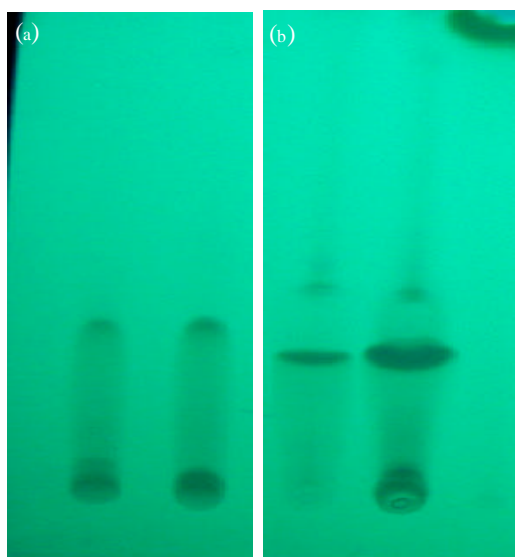


Fig. 2(a-b): Revelation of TLC plate with UV of methanolic (a) and ethanolic (b) extracts in pre-flowering (a) and flowering (b) stages

the number of separated compounds of extracts. Moreover, exact analysis of phenolic compound performed by SPE-reversed phase-HPLC method. SPE clean-up using ethyl silica gel cartridge was applied in order to pre-concentrate, remove interfering compounds and increase the efficiency of HPLC. The water-methanol-acetone extract in flowering stage was fractionated with buffer phosphate (pH = 5, 6, 7, 8.5, 10, 11.5) as washing buffer. Antioxidant activities in collected fractions were determined with DPPH reagent. The fraction collected with washing buffer (pH = 6) showed the highest antioxidant activity. This is in agreement with the results reported by Matthaus (2007). In his review article, Matthaus has explained that washing buffer with pH = 6-7 is effective for maximum separation of phenolic compounds of resin. This fraction and the extract before SPE clean-up were then injected to HPLC (Fig. 3). Figure 3 is a typical representative of the HPLC chromatogram for only the flowering stages. These findings were compared with the results of the extracts which were directly injected into column.

On the other hand, Kim and Lee (2004) have reported that rosmarinic acid is the main antioxidant in the Labiatae family. Many other authors have evaluated the content of rosmarinic acid in peppermint, spearmint, rosemary, sage (Diandian, 2008). The results obtained by Diandian (2008) have indicated that rosmarinic acid is the major phenolic acid in the HPLC profile of 50% ethanolic extract from mint. We, therefore, decided to determine the concentration of rosmarinic acid in water-methanol-acetone extract from both stages. Using the serial dilutions of stoke standard solution ($20 \mu\text{g mL}^{-1}$), the calibration curves for rosmarinic acid in the range of $0.5\text{-}20 \mu\text{g mL}^{-1}$ at five concentration levels was obtained. Calibration plot was obtained by reporting peak areas as a function of analyte concentrations ($R^2 = 0.998$). It was found that the concentration of rosmarinic acid in the extract of pre-flowering and flowering period were 130 and $120 \mu\text{g } 100 \text{ g}^{-1}$ dry weight, respectively. Our results showed that the level of rosmarinic acid in the samples in pre-flowering stage was higher than the samples in flowering stage. This result supported by similar studies about the effect of environment on accumulation of RA in *M. piperita* and *M. spicata* experienced by Fletcher *et al.* (2010). The author reported that concentration of RA in pre-flowering stage were greater than flowering stage. Their findings are in agreement with those reported by Papageorgiou *et al.* (2008) and both reports support our findings from this study. The physiological stages of the samples have the major role in accumulation of RA. The investigation done by Fletcher has shown that by entering the flowering stage, RA content began to

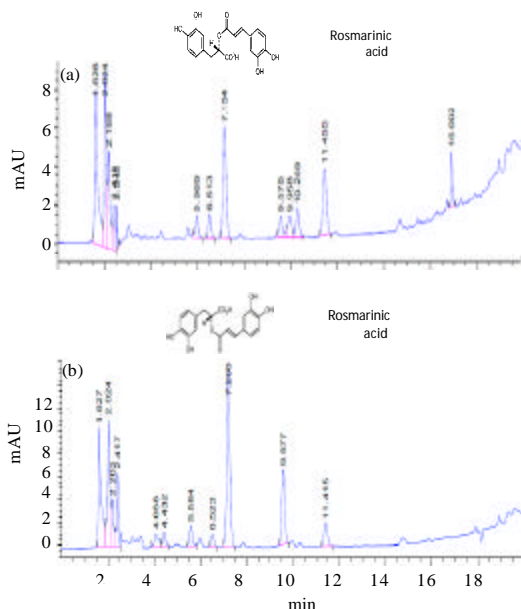


Fig. 3(a-b): Typical HPLC chromatogram of water-methanol-acetone extract in flowering stage (a) before and (b) after SPE clean-up

decrease. They also found that the samples in the vegetative period had the highest amount RA. According to the results obtained by Fletcher *et al.* (2010), when the plant moves from the physiological state towards reproduction, it may reallocate resources from RA production to the reproductive phase. Most probably it draws phenolics towards the production of lignin and formation of suberin in order to strengthen stem for structural integrity and to flavonoid production for flowering. This must be a usual mechanism in the Lamiaceae family. Therefore, it can be recommended that in commercial purposes, it is more useful to harvest the plants before flowering stage to get the optimum RA of the leaves of *M. pulegium*.

CONCLUSION

The results obtained from this survey demonstrated that the extracts of *M. pulegium* in flowering season possess higher phenolic compounds and antioxidant capacity than the extracts in pre-flowering season. Moreover, the hydroalcoholic solvents extracted the highest amount of polyphenols due to the polarity of phenolic compounds. It could be emphasized that the physiological stages of the plant possess a basic role in accumulation of phenolic acids. On the other hand, the positive relationship between phenol contents and antioxidant behavior shows the importance of the

phenolic compounds in the antioxidant activity of *M. pulegium* extracts. The TLC chromatograms of extracts showed that using of solvents with different polarity leads to extraction of various secondary metabolites. Our finding approved that SPE clean-up is a suitable procedure for concentration of samples and removal of interfering compounds before injection to HPLC. It is concluded that *M. pulegium* obtained from Gilan province in northern Iran is potentially a rich natural source of antioxidant, especially during its flowering season. Furthermore, our investigations suggested that this herb could be used as an excellent natural preservative in food and pharmaceutical industries. However, more extensive studies are required for identification and isolation of their major antioxidant compounds.

ACKNOWLEDGMENTS

The financial support by University of Guilan is highly appreciated. We also express our sincere thanks to Professor Ali Akbar for his helpful scientific advices.

REFERENCES

- Ali, S.S., N. Kasoju, A. Luthra, A. Singh, H. Sharanabasava, A. Sahu and U. Bora, 2008. Indian medicinal herbs as sources of antioxidants. *Food Res. Int.*, 41: 1-15.
- Atmami, D., N. Chaher, M. Berboucha, K. Ayouni and H. Lounis *et al.*, 2009. Antioxidant capacity and phenol content of selected algerian medicinal plants. *Food Chem.*, 112: 303-309.
- Benzie, I.F. and J.J. Strain, 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.*, 299: 15-27.
- Bolton, J.L., M.A. Trush, T.M. Penning, G. Dryhurst and T.J. Monks, 2000. The role of quinones in toxicology. *Chem. Res. Toxicol.*, 13: 135-160.
- Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.*, 28: 25-30.
- Capecka, E., A. Mareczek and M. Leja, 2005. Antioxidant activity of fresh and dry herbs of some Lamiaceae species. *Food Chem.*, 93: 223-226.
- Carnat, A., D. Fraisse, A.P. Carnat, C. Felgines, D. Chaud and J.L. Lamaison, 2005. Influence of drying mode on iridoid bitter constituent levels in gentian root. *J. Sci. Food Agric.*, 85: 598-602.
- Chalchat, J.C., M.S. Gorunovic, Z.A. Maksimovic and S.D. Petrovic, 2000. Essential oil of wild growing *Mentha pulegium* L from Yugoslavia. *J. Essential Oil Res.*, 12: 598-600.

- Diandian, S., 2008. Development of anti-inflammatory agents from the aromatic plants, *Origanum* ssp and *Mentha* spp. and analytical methods on the quality control of bioactive phenolic compounds. Ph.D. Thesis, The State University of New Jersey, Rutgers, New Jersey.
- Fletcher, S.R., T. Slimmon and S.L. Kott, 2010. Environmental factors affecting the accumulation of rosmarinic acid in spearmint (*Mentha spicata* L.) and peppermint (*Mentha piperita* L.). Open Agric. J., 4: 10-16.
- Gao, X., M. Ohlander, N. Jeppsson, L. Bjork and V. Trajkovski, 2000. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. J. Agric. Food Chem., 48: 1485-1490.
- Ghasemnezhad, M., M. Sherafati and G.A. Payvast, 2011. Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. J. Funct. Foods, 3: 44-49.
- Guidi, I., D. Galimberti, S. Lonati, C. Novembrino and F. Bamonti *et al.*, 2006. Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. Neurobiol. Aging, 27: 262-269.
- Hai, W., H. Cheng, M. Zhao, Y. Wang, L. Hong, H. Tang and X. Tian, 2012. Two new cytotoxic triterpenoid saponins from the roots of *Clematis argentea*. Fitoterapia, 83: 759-764.
- Ismail, A., Z.M. Marjan and C.W. Foong, 2004. Total antioxidant activity and phenolic contents in selected vegetables. Food Chem., 87: 581-586.
- Ito, N., S. Fukushima, A. Hasegawa, M. Shibata and T. Ogiso, 1983. Carcinogenicity of butylated hydroxyanisole in F344 rats. J. Nat. Cancer Inst., 70: 343-347.
- Kamkar, A., A.J. Javan, F. Asadi and M. Kamalinejad, 2010. The antioxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil. Food Chem. Toxicol., 48: 1796-1800.
- Katalinic, V., M. Milos, T. Kulisic and M. Jukic, 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem., 94: 550-557.
- Kim, D.O. and C.Y. Lee, 2004. Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. Crit. Rev. Food Sci. Nutr., 44: 253-273.
- Kosar, M., H.J.D. Dorman and R. Hiltunen, 2005. Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected *Lamiaceae* species. Food Chem., 91: 525-533.
- Kris-Etherton, P.M., K.D. Hecker, A. Bonanome, S.M. Coval and A.E. Binkoski *et al.*, 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am. J. Med., 30: 71S-88S.
- Kumar, K.V. and D.D. Patra, 2012. Alteration in yield and chemical composition of essential oil of *Mentha piperita* L. plant: Effect of fly ash amendments and organic wastes. Ecol. Eng., 47: 237-241.
- Li, J.W., S.D. Ding and X.L. Ding, 2005. Comparison of antioxidant capacities of extracts from five cultivars of Chinese jujube. Process Biochem., 40: 3607-3613.
- Mahboubi, M. and G. Haghi, 2008. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. J. Ethnopharmacol., 119: 325-327.
- Matkowski, A., P. Tasarz and E. Szypuła, 2008. Antioxidant activity of herb extracts from five medicinal plants from Lamiaceae, subfamily Lamioideae. J. Med. Plant Res., 2: 321-330.
- Matthaus, B., 2007. Oil technology. Adv. Botanical Res., 45: 483-527.
- Moure, A., D. Franco, J. Sineiro, H. Domínguez, M.J. Nunez and J.M. Lema, 1999. Antioxidant properties of *Gevuina avellanahulls*. Proceedings 8th Mediterranean Congress of Chemical Engineering, November 10-12, 1999, Barcelona, Spain.
- Mozaffarian, V., 1996. A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran, pp: 198.
- Nickavar, B., A. Alinaghi and M. Kamalinejad, 2008. Evaluation of the antioxidant properties of five *Mentha* species. Iranian J. Pharm. Res., 7: 203-209.
- Papageorgiou, V., C. Gardeli, A. Mallouchos, M. Papaioannou and M. Komaitis, 2008. Variation of the chemical profile and antioxidant behavior of *Rosmarinus officinalis* L. and *Salvia fruticosa* miller grown in Greece. J. Agric. Food Chem., 56: 7254-7264.
- Rahmat, A., V. Kumar, L.M. Fong, S. Endrini, H. Abdullah Sami, 2003. Determination of total antioxidant activity in three types of local vegetables shoots and the cytotoxic effect of their ethanolic extracts against different cancer cell lines. Asia Pacific J. Clin. Nutr., 12: 308-311.
- Shahmohamadi, R., R. Sariri, M. Rasa, H. Ghafoori, M. Aghamali, S. Nasuti and M. Tahery, 2011. Chemical composition and antimicrobial activity of flowering aerial parts *Mentha Pulegium* from Gilan. Pharmacologyonline, 3: 651-659.
- Shirazi, F.H., N. Ahmadi and M. Kamalinejad, 2004. Evaluation of northern Iran *Mentha pulegium* L. Cytotoxicity. Daru, 12: 106-110.

- Smith, M.A., C.A. Rottkamp, A. Nunomura, A.K. Raina and G. Perry, 2000. Oxidative stress in Alzheimer's disease. *Biochim. Biophys. Acta*, 1502: 139-144.
- Spigno, G., L. Tramelli and D.M. De Faveri, 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng.*, 81: 200-208.
- Sultana, B., F. Anwar and R. Pryzbylski, 2007. Antioxidant activity of phenolic components present in barks of *Azadiractha indica*, *Terminalia arjuna*, *Acacia nilotica* and *Eugenia jambolana* Lam. Trees. *Food Chem.*, 104: 1106-1114.
- Tepe, B., D. Daferera, A. Sokmen, M. Sokmen and M. Polissiou, 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chem.*, 90: 333-340.
- Van Horssen, J., G. Schreibelt, J. Drexhage, T. Hazes, C.D. Dijkstra, P. Van der Valk and H.E. De Vries, 2008. Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. *Free Radical Biol. Med.*, 45: 1729-1737.
- Wang, J., X. Yuan, Z. Jin, Y. Tian and H. Song, 2007. Free radical and reactive oxygen species scavenging activities of peanut skins extract. *Food Chem.*, 104: 242-250.
- Wojdylo, A., J. Oszmianski and R. Czemerys, 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.*, 105: 940-949.
- Zargari, A., 1990. Herbal Medicines. 1st Edn., Tehran Publication of Tehran University, Iran.