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# Studies on free-radical scavenging activity and identification of active ingredients of different plant crude extracts of *Mentha piperita* collected from Sur, Sultanate of Oman

Mohammad Amzad Hossain[1*], Aqeela Said Hamed AL Orimi[1], Afaf Mohammed Weli[1], Qasim Al-Riyami[1], Jamal Nasser Al-Sabahi[2]

[1]*School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P. O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman*

[2]*Central Instrument Laboratory, College of Agricultural and Marine Sciences, Sultan Qaboos University, Sultanate of Oman*

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ABSTRACT

**Objective:** To determine free radical scavenging activity and active chemical ingredients of different plant crude extracts of *Mentha piperita* (*M. piperita*).

**Methods:** The dried powder leaves of *M. piperita* were extracted with polar organic solvent by Soxhlet extractor. The crude extract and its fractions of hexane, chloroform, ethyl acetate and butanol crude extracts were prepared. The antioxidant activity of different crude extracts from *M. piperita* was carried out by DPPH method with minor modification, and the active chemical ingredients of different plant crude extracts of *M. piperita* were analyzed by gas chromatography-mass spectrometry (GC-MS).

**Results:** Qualitative analysis of different polarities crude extracts by GC-MS found different types of active organic compounds. The antioxidant activity of different crude extracts were found to be in the order of chloroform extract> butanol extract> ethyl acetate extract> hexane extract> methanol extract. Majority identified compounds in the plant crude extracts by GC-MS were biologically active.

**Conclusions:** Therefore, the isolation, purification, identification and characterization of bioactive compounds from various crude extracts of *M. piperita* might have ecological significance.

## 1. Introduction

*Mentha piperita* L. (*M. piperita*) is the most important traditional medicinal plants available worldwide. It is also found in the Sultanate of Oman. Locally it is known as peppermint belonging to the family of Lamiaceae. Traditionally, this plant is used by different ethnic communities of Sultanate of Oman for the treatment of cough and other cough related diseases[1]. It is native to European countries and Asia. Some varieties of *M. piperita* are indigenous to South Africa, South America, and Australia.Now it is a hybrid plant and cross between watermint and spearmint[2]. This plant grows well in all environmental conditions and the height is about 2-3 feet. It comes into flower from July through August,

*Corresponding author: Dr. Mohammad A. Hossain, School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P. O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman.

Tel: +96892327578

Fax: +96892877745

E-mail: hossainabi@gmail.com

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sprouting tiny purple flowers in whorls and terminal spikes. Dark green fragrant leaves grow opposite white flowers[3]. The whole plant is used as a flavoring agent for the preparation of food, gum, toothpaste and tea[4]. It is also used to soothe upset stomach and to aid digestion[5]. The crude extracts from *M. piperita* have been used as the therapeutic treatment of headaches, skin irritations, anxiety associated with depression, nausea, diarrhea, menstrual cramps and flatulence[4]. *In vitro* test shows that *M. piperita* crude extracts kill some types of bacteria, fungus and viruses. It suggests that it may have antibacterial, antifungal, and antiviral properties[6,7].

Several scientific studies supported the use of *M. piperita* crude extract for indigestion and irritable bowel syndrome[8]. Recently, it has been also used to relax the muscles that allow painful digestive gas to pass[9-11]. The reported active main ingredient of *M. piperita* is menthol, which is an effective decongestant[9-11]. The menthol is also a good expectorant, meaning that it helps loosen phlegm and breaks up coughs[12-15].

The previous study mentioned that the leaves and stems contained high concentrations of menthol type compounds that are used medicinally as a flavoring agent in food and cosmetics[16-18]. The literature search reveals that still no research has been done on Omani *M. piperita*. In addition, the available information with regard to correlating the phytochemical constituents and other activities of this plant is lacking. Therefore, the present study is to prepare different crude extracts collected from locally grown species and to identify their active chemical ingredients by gas chromatography-mass spectrometry (GC-MS) and evaluate their free radical scavenging activity by DPPH method.

## 2. Materials and methods

### 2.1. Sample collection

The healthy whole plants of *M. piperita* were collected from garden at Sur, Sultanate of Oman. The plants were harvested during the month of April 4, 2012 at 8 am. The collected samples were packed in a polyethylene bag and stored at 4 °C.

### 2.2. Preparation of samples

The collected plant samples were washed with fresh water to remove all dust. The whole plant samples were dried under shade for complete dry. Approximately 60 g of plant samples were ground using a grinder (Super Deluxe, India) for 20 seconds. The small pieces of samples were homogenized in a grinder for 3 min to 40-60 mesh size. The dried plant sample was pulverized into powdered form.

### 2.3. Preparation of crude extracts

The powder sample (60 g) was extracted with methanol (200 mL, 72 h) by using a Soxhlet extractor. The solvent was evaporated by rotary evaporator (Yamato, Rotary Evaporator, Model-RE 801) to give gummy paste weight about 7.82 g. The 7 g crude extract was suspended in 100 mL of distilled water. Then it was extracted successively with hexane, chloroform, ethyl acetate and butanol to derive hexane (1.05 g), ethyl acetate (1.47 g), chloroform (0.37 g), butanol (1.45 g) and residual methanol fractions (2.13 g), respectively. The plant residue was re-extracted twice following the same procedure and filtered. The combined extracts were concentrated by using rotary evaporator and dried under vacuum.

### 2.4. Radical scavenging activity using DPPH method

Free radical scavenging activity of different crude extracts with different polarities of *M. piperita* was estimated by the method described by Hossain with minor modification[19]. The different crude extracts of *M. piperita* (4 mL) in different concentrations (12.5, 25, 50, 100 and 200 mg/L, respectively) were taken in different test tubes. One milliliter of 0.1 mmol/L DPPH was dissolved in methanol, added to those tubes, and shaken vigorously. After shaking, all the test tubes were allowed to stand at 27 °C in a dark place for 45 min. The blank control was prepared in the same way without extract. The differences in the absorbance of the prepared samples were measured using UV spectroscopy at 517 nm. Radical scavenging activity of the tested different crude extracts was estimated as an inhibition percentage (%) and calculated by using the following formula,

Measurement of radical scavenging activity (%)

$$\% \text{ Inhibition} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

### 2.5. GC-MS analysis

The GC-MS analysis of different polarities crude extracts from whole plant of *M. piperita* was performed using a Perkin Elmer Clarus 600 GC system equipped with a Rtx®-5MS fused silica capillary column (30 m×0.25 mm i.d., film thickness 0.25 μm). The GC system was coupled with a Perkin Elmer Clarus 600 MS. Gas chromatography-mass spectroscopic detector of an electron ionization mode with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Mass transfer line and injector temperatures were set at 260 and 290 °C, respectively. The oven was programmed from 60 °C (hold 2 min) to 270 °C at 4 °C/min, then held isothermal for 20 min and finally raised to 290 °C at 10 °C/min. Diluted samples (1/100, v/v, in methanol) of 1 μL were injected in the split mode with a split ratio of 120:1. The calculation of the percentage (%) of the crude extracts constituents was expressed as a percentage by peak area normalization.

### 2.6. Identification of chemical constituents

The chemical ingredients in different crude extracts from the *M. piperita* were identified based on GC retention time on Rtx®-5MS fused silica capillary column, computer matching of mass spectra with those of standards (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003 of GC-MS systems)[19].

## 3. Results

The samples of *M. piperita* were extracted with methanol by Soxhlet extractor and the solvent was evaporated by rotary evaporator to give a gummy paste. The percentage (%) yields of extraction from the whole plants powder of *M. piperita* were hexane, ethyl acetate, chloroform, butanol and residual methanol fractions, respectively.

### 3.1. Physical properties

The different crude extracts from the whole plant have different colors. The hexane crude extract was deep brown in color, ethyl acetate was pale yellow in color, chloroform extract was deep orange and the butanol extract was blackish in color.

### 3.2. Chemical composition of different extracts

The hexane crude extract from the whole plant of local *M. piperita* was analyzed by using GC-MS. It contains 22 different organic compounds, representing 1.52% of the total extract. The identification of the active chemical compounds was done by GC-MS listed in Table 1 according to their elution order on Rtx®-5MS capillary column. The major active ingredients that were identified in hexane extract (shown in Table 1 and Figure 1) are pulegone (1.11%), carvone (9.76%), verbenone (2.79%), bicycle [7.2.0] undec-4-ene, eupatorin (2.12%), (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol (4.68%), hexahydrofarnesyl acetone (2.04%), (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.53%), methyl 14-methylpentadecanoate (4.14%), methyl linolenate (8.15%), phytol (24.16%), butyl palmitate (6.90%), 2,6,10,15-tetramethylheptadecane (2.90%), 1-teracosanol (1.31%), teracosanol (4.36%), isobutyl stearate (4.27%), eicosane, 7-hexyl- (11.47%) and nonacosane (8.19%).

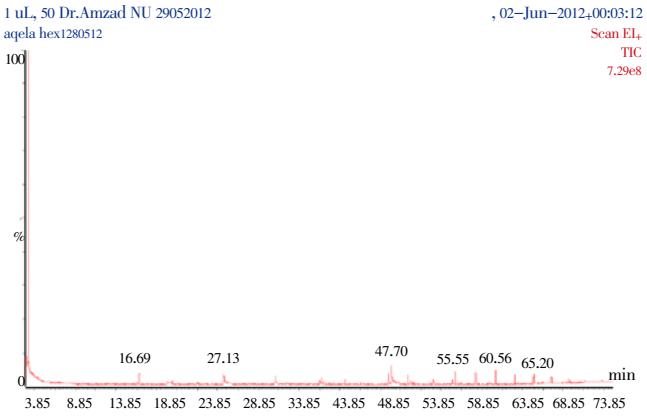


**Figure 1.** A typical gas chromatogram of the chemical constituents of hexane crude extract.

The ethyl acetate crude extract was analyzed by using GC-MS leading to the identification of a total of 7 different organic compounds using the same capillary column and conditions, representing 1.19% of the total extract from whole plant. The major chemical constituents that were identified in ethyl acetate crude extract by GC-MS (Figure 2 and Table 1) are carvone (6.47%), 3-eicosyne (2.85%), methyl 14-methylpentadecanoate (8.55%), (9E,12E,15E)-9,12,15-octadecatrien-1-ol (1.37%), sinensetin (1.42%), methyl linolenate (20.77%), phytol (54.18%) and methyl heptacosanoate (2.36%).

**Table 1**

Chemical composition of different organic plant crude extracts of peppermint.

| Name of compounds | Retention time (min) | Molecular formula | Leave (%) |
|---|---|---|---|
| Hexane crude extract | | | |
| Pulegone | 16.510 | $C_{10}H_{16}O$ | 0.8100 |
| (+)-Carvone | 16.690 | $C_{10}H_{14}O$ | 7.0910 |
| Verbenone | 20.700 | $C_{10}H_{14}O$ | 2.0290 |
| Caryophyllene | 23.900 | $C_{15}H_{24}$ | 1.5750 |
| 3-Eicosyne | 39.160 | $C_{20}H_{40}O$ | 3.4000 |
| Hexahydrofarnesyl acetone | 39.370 | $C_{18}H_{36}O$ | 1.4880 |
| 3-Eicosyne | 40.550 | $C_{20}H_{40}O$ | 2.1240 |
| Methyl 14-methylpentadecanoate (FA) | 42.020 | $C_{17}H_{34}O_2$ | 3.0110 |
| Methyl linolenate (FA) | 47.340 | $C_{19}H_{32}O_2$ | 5.9230 |
| Phytol | 47.700 | $C_{20}H_{40}O$ | 17.5520 |
| Butyl palmitate (FA) | 49.810 | $C_{20}H_{40}O_2$ | 5.0130 |
| Docosane | 49.990 | $C_{21}H_{44}$ | 2.1070 |
| Octadecanol acetate | 50.480 | $C_{24}H_{50}O$ | 0.9540 |
| Tricosane | 52.910 | $C_{24}H_{50}$ | 3.1680 |
| Isobutyl stearate (FA) | 55.280 | $C_{22}H_{44}O_2$ | 3.1050 |
| Tetracosane | 55.550 | $C_{26}H_{54}$ | 8.0750 |
| Pentacosane | 58.110 | $C_{29}H_{60}$ | 7.0620 |
| Hexacosane | 60.560 | $C_{22}H_{44}O_2$ | 8.3330 |
| Heptacosane | 62.920 | $C_{28}H_{48}$ | 5.7440 |
| Octacosane | 65.200 | $C_{34}H_{50}O$ | 5.9510 |
| Nonacosane | 67.430 | $C_{35}H_{52}$ | 3.4180 |
| Triacontane | 69.560 | $C_{32}H_{54}O_2$ | 3.0740 |
| Ethyl acetate extract | | | |
| Carvone | 16.624 | $C_{10}H_{14}O$ | 6.7000 |
| 3-Eicosyne | 39.149 | $C_{20}H_{38}$ | 2.9600 |
| Methyl 14-methylpentadecanoate (FA) | 41.995 | $C_{17}H_{34}O_2$ | 8.8600 |
| Sinensetin | 44.131 | $C_{18}H_{32}O$ | 1.4270 |
| Methyl linolenate (FA) | 47.327 | $C_{19}H_{32}O_2$ | 21.5080 |
| Phytol | 47.682 | $C_{20}H_{40}O$ | 56.0940 |
| Methyl isoheptadecanoate (FA) | 48.117 | $C_{28}H_{56}O_2$ | 2.4500 |
| Chloroform extract | | | |
| Eupatorin | 39.159 | $C_{20}H_{40}O$ | 18.0483 |
| Methyl 14-methylpentadecanoate (FA) | 42.020 | $C_{17}H_{34}O_2$ | 40.7430 |
| Methyl linolenate (FA) | 47.333 | $C_{18}H_{31}OCl$ | 14.9235 |
| Phytol | 47.700 | $C_{18}H_{36}O_2$ | 11.7032 |
| Methyl isoheptadecanoate (FA) | 48.123 | $C_{20}H_{40}O_2$ | 14.5820 |
| Butanol extract | | | |
| o-Tolualdehyde | 15.744 | $C_8H_8O$ | 60.1880 |
| Methyl 14-methylpentadecanoate (FA) | 42.035 | $C_{17}H_{34}O_2$ | 39.8110 |
| Methanol crude extract | | | |
| Pulegone | 16.514 | $C_{10}H_{16}O$ | 7.2040 |
| Carvone | 16.704 | $C_{10}H_{14}O$ | 40.4600 |
| Eugenol | 21.416 | $C_{10}H_{12}O_2$ | 9.0740 |
| 3-Eicosyne | 39.170 | $C_{17}H_{34}O_2$ | 7.7970 |
| Methyl 14-methylpentadecanoate (FA) | 42.030 | $C_{18}H_{31}OCl$ | 18.0740 |
| Methyl linolenate (FA) | 47.347 | $C_{17}H_{34}O_2$ | 17.3900 |

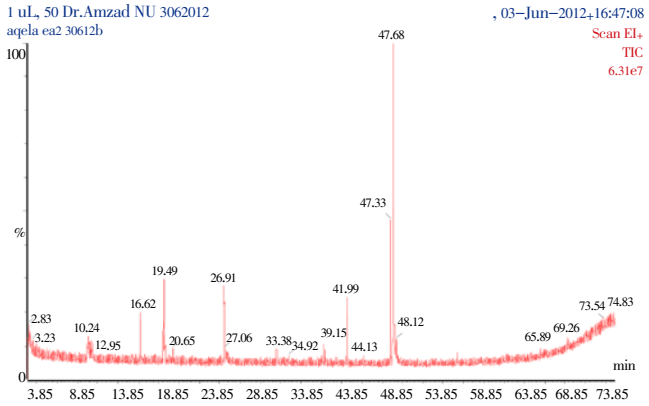


**Figure 2.** A typical gas chromatogram of the chemical constituents of ethyl acetate crude extract.

The chloroform crude extract was analyzed by using GC-MS resulting in the identification of 4 different organic compounds using the same capillary column and conditions, representing 0.88% of the total extract. The chemical constituents that were found in chloroform plant crude extract (Figure 3 and Table 1) are identified as (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol (20.44%), methyl 14-methylpentadecanoate (46.14%), linoleoyl chloride (16.90%) and methyl isoheptadecanoate (16.51%).

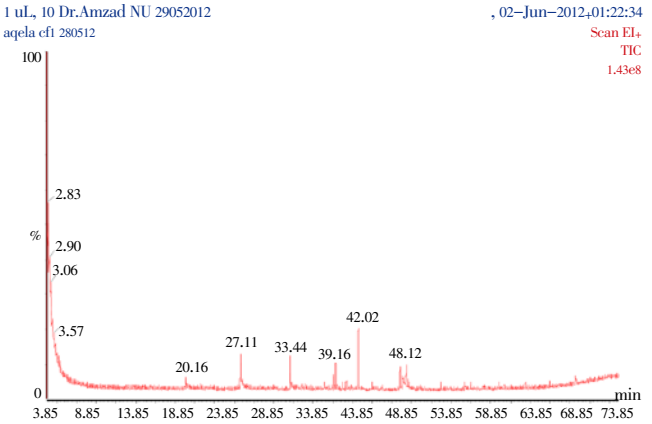


**Figure 3.** A typical gas chromatogram of the chemical constituents of chloroform crude extract.

The butanol plant crude extract was analyzed by using GC-MS, leading to the identification of 2 different organic compounds, representing 0.69% of the total extract. The major chemical constituents found in butanol crude extract (Figure 4 and Table 1) are o-tolualdehyde (27.97%) and methyl 14-methylpentadecanoate (18.50%).

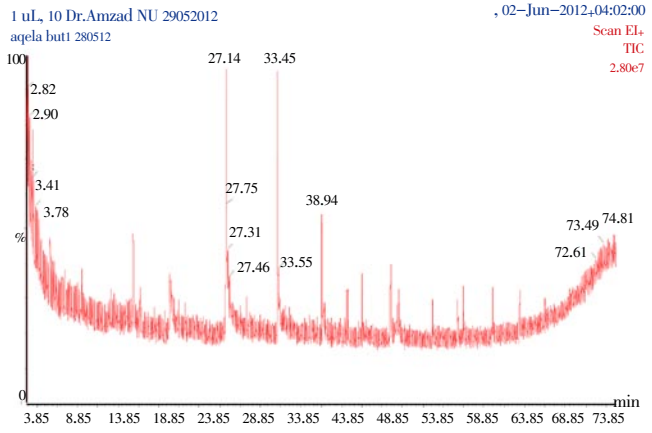


**Figure 4.** A typical gas chromatogram of the chemical constituents of butanol crude extract.

Finally the methanol crude extract was analyzed using GC-MS leading to identification of 5 different organic compounds representing 2.13% of the total extract. The major chemical constituents identified in methanol plant crude extract (Figure 5 and Table 1) are pulegone (6.00%), carvone (33.72%), eugenol (7.56%), methyl 14-methylpentadecanoate (15.06%) and lineoleoyl chloride (14.49%).

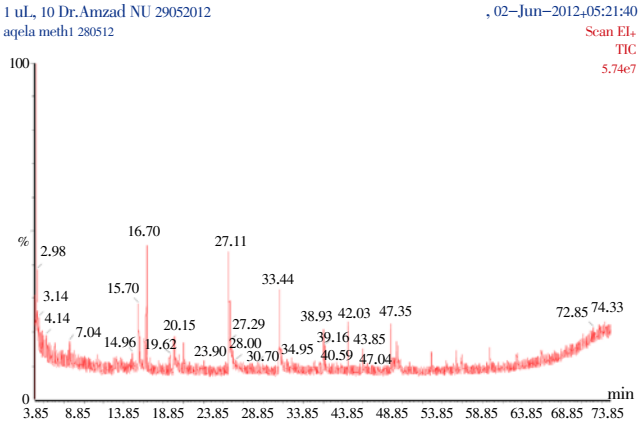


**Figure 5.** A typical gas chromatogram of the chemical constituents of methanol crude extract.

The free radical scavenging activity of different polarities crude extracts was tested through DPPH method and the results are presented in Figure 6. The antioxidant activity in their interaction depends on oxidative free radicals. The mechanism of DPPH method is that the antioxidants react with the stable free radical *i.e.*, α,α-diphenyl-β-picrylhydrazyl (deep violet colour) and convert it to α,α-diphenyl-β-picrylhydrazine with discoloration. The gradual discoloration indicates the scavenging capacities of the plant crude extracts. In the present study, the five different plant crude extracts extracted from locally grown *M. piperita* were able to decolorize DPPH and the free radical scavenging capacities of the plant crude extracts were found to be in the order of chloroform> butanol> ethyl acetate extract> hexane extract> methanol (Figure 6). The present experiment used DPPH as a control. It has been found that all aromatic and aliphatic organic compounds such as aromatic hydrocarbon, cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy and other aromatic amines such as *p*-phenylene diamine, *p*-aminophenol *etc*., gradually reduce and decolorize by α,α-diphenyl-β-picrylhydrazyl revealing their free radical ability[19,20]. In this study, it appears that perhaps the five different crude extracts from local *M. piperita* possess strong hydrogen donating capabilities to act as antioxidants.

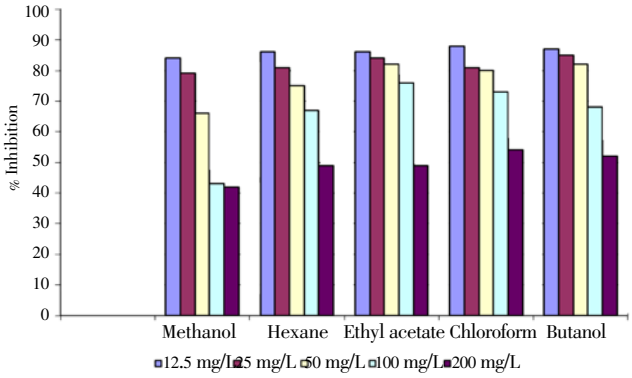


**Figure 6.** Free radical scavenging activity of peppermint crude extracts by DPPH methods.

## 4. Discussion

The different crude extracts of local *M. piperita* for respective bioactive compounds can be chosen on the basis of the above GC-MS analysis. Phytol is a major terpenoid found to be the most abundant in the hexane, ethyl acetate and chloroform crude extract. Hexane crude extract contains a lot of normal and cyclic hydrocarbons with high molecular weight. Ethyl acetate crude extract also contains methyl linolenate, phytol and some other aliphatic and aromatic chemical compounds such as flavonoids. Chloroform crude extract contains flavonoids eupatorin, (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol and methyl 14-methylpentadecanoate; butanol crude extract contains o-tolualdehyde and methyl 14-methylpentadecanoate and methanol crude extract contains carvone, methyl 14-methylpentadecanoate and lineoleoyl chloride. All the major chemical compounds have previously been reported from a number of other plant species[19,20]. The major chemical compounds identified from different crude extracts by GC-MS are basically biologically active molecules reported in different plants crude extracts by the researchers[5,8,10-18]. All the predominant compounds in this plant crude extracts such as sinensetin, euparotin *etc.* are considered to be a part of plants' defense system, and as such have been included in a large group of protective molecules found in plants named 'phytoanticipins' or 'phytoprotectants'[17-19]. Therefore, the identified chemical compounds from various crude extracts of *M. piperita* by GC-MS might have some ecologically significant role. So far as known, this report is the first report to investigate *in vitro* antioxidant activities as well as chemical composition of different crude extracts of locally grown *M. piperita* using GC-MS. Hence, the entire *M. piperita* plant could be of use as a good source of antioxidant. Further studies are needed for the isolation and identification of individual bioactive compounds from the plant crude extracts of *M. piperita* and also *in vivo* studies are needed for better understanding of their mechanism of action as antioxidant.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## References

[1] Ahmeda A, Hossain MA, Ismail Z. Antioxidant properties of the isolated flavonoids from the medicinal plant *Phyllanthus niruri*. *Asian J Food Ag-Ind* 2009; **2**: 373-381.

[2] Sharafi SM, Rasooli I, Owlia P, Taghizadeh M, Astaneh SD. Protective effects of bioactive phytochemicals from *Mentha piperita* with multiple health potentials. *Pharmacogn Mag* 2010; **6**(23): 147-153.

[3] Stojanova A, Paraskevova P, Anastassov C. A comparative investigation on the essential oil composition of two Bulgarian cultivars of *Mentha piperita* L. *J Essen Oil Res* 2000; **12**: 438-440.

[4] Chessa M, Sias A, Piana A, Mangano GS, Petretto GL, Masia MD et al. Chemical composition and antibacterial activity of the essential oil from *Mentha requienii* Bentham. *Nat Prod Res* 2013; **27**: 93-99.

[5] Gardiner P. Peppermint (*Mentha piperita* ). The Longwood Herbal Task Force and the Center for Holistic Pediatric Education and Research; 2000. [online] Available from: http://www.longwoodherbal.org/peppermint/peppermint.pdf [Accessed on 20th June 2014]

[6] Nickavar B, Alinaghi A, Kamalinejad M. Evaluation of the antioxidant activity of five *Mentha* species. *Iranian J Pharm Res* 2008; **7**(3): 203-209.

[7] Singh R, Shushni MA, Belkheir A. Antibacterial and antioxidant activities of *Mentha piperita* L. *Arabian J Chem* 2011; doi: 10.1016/j.arabjc.2011.01.019.

[8] Derwich E, Chabir R, Taouil R. *In vitro* antioxidant activity and GC-MS studies on the leaves of *Mentha piperita* (Lamiaceae) from Morocco. *Int J Pharm Sci Drug Res* 2011; **3**(2): 130-136.

[9] Mimica-Dukić N, Bozin B, Soković M, Mihajlović B, Matavulj M. Antimicrobial and antioxidant activities of three Mentha species essential oils. *Planta Med* 2003; **69**: 413-419.

[10] Kizil S, Hasimi N, Tolan V, Kilinc E, Yuksel U. Mineral content, essential oil components and biological activity of two menthe species (*M. Piperita* L., *M. Spicata* L.). *Turk J Field Corps* 2010; **15**: 148-153.

[11] Grulova D, Labun P, Sersen F, Salamon I. Seasonal variation in DPPH scavenging activity of *Mentha piperita*. *Adv Environ Biol* 2012; **6**(4): 1477-1480.

[12] Promila DM, Xavier R, Marimuthu K, Kathiresan S, Khoo ML, Senthilkumar M, et al. Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (*Mentha piperita*: Lamiaceae). *J Med Plant Res* 2012; **6**(2): 331-335.

[13] Atanassova M, Georgieva S, Ivancheva K. Total phenol and total flavonoid contents, antioxidant capacity and biological contaminanta in medicinal herbs. *J Univ Chem Technol Metall* 2011; **46**(1): 81-88.

[14] Al Nomaani RS, Hossain MA, Weli AM, Al-Riyami Q, Al-Sabahi JN, Rahman SM. Chemical composition of essential oils and *in vitro* antioxidant activity of fresh and dry leaves crude extracts of medicinal plant of *Lactuca sativa* L. native to Sultanate of Oman. *Asian Pac J Trop Biomed* 2013; **3**(5): 353-357.

[15] Hossain MA, Shah MD. Studied on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant *Merremia borneensis*. *Arabian J Chem* 2011; doi: 10.1016/j.arabjc.2011.01.007.

[16] Hossain MA, Shah MD, Gnanaraj C, Iqbal M. *In vitro* total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant *Tetrastigma* from Sabah. *Asian Pac J Trop Med* 2011; **4**(9): 717-721.

[17] Hossain MA, Rahman SM. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. *Food Res Int* 2011; **44**: 672-676.

[18] Dev N, Das AK, Hossain MA, Rahman SM. Chemical compositions of different extracts of *Ocimum basilicum* leaves. *J Sci Res* 2011; **3**(1): 197-206.

[19] Hashmi LS, Hossain MA, Weli AM, Al-Riyami Q, Al-Sabahi JN, Rahman SM. Gas chromatography-mass spectrometry analysis of different organic crude extracts from the local medicinal plant of *Thymus vulgaris* L. *Asian Pac J Trop Biomed* 2013; **3**(1): 69-73.

[20] Tabari MA, Youssefi MR, Ghasemi F, Tabari RG, Esmaili RH, Behzadi MY. Comparison of antibacterial effects of *Eucalyptus* essence, mint essence and combination of them on *Staphylococcus aureus* and *Escherichia coli* isolates. *Middle-East J Sci Res* 2012; **11**(4): 536-540.