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# Phytochemistry and Antioxidant Activity of Essential Oils of Condiment and Spice Plants from South Western, Iran

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**Abstract:** Essential oils and volatile constituents have a wide range of applications in folk medicine, food flavoring and food preservation as well as in food industries. The phytochemical characteristics of hydro-distillation essential oils from different parts of Iranian aromatic, condiment and spice plants belonging to the families Lamiaceae and Apiaceae including *Satureja bachtiarica*, *S. khuzestanica*, *S. hortensis*, *Thymus daenensis*, *T. carmanicus*, *Ziziphora clinopodioides*, *Z. teniuor* and *Mentha longifolia* (Lamiaceae), *Echinophora cinerea*, *E. platyloba*, *Heracleum lasiopetalum*, *Kelussia odoratissimay*, *Zaravschanica membranacea*, *Cuminum cyminum* and *Ferulago angulata* (Apiaceae) were analyzed by GC and GC/MS. The antioxidant activity of the essential oils from the herbs was evaluated by DPPH assay. Results indicated that the main constituents of the herbs were monoterpenes in mostly herbs. Other identified chemical components in the essential oils from *H. lasiopetalum* and *K. odoratissima* were n-octanol and trans-ligustilide, respectively. The essential oils from the studied herbs had weak-to-good antioxidant activity. Among the herb species screened, the essential oils from *S. bachtiarica*, *S. khuzestanica*, *S. hortensis*, *Thymus daenensis* and *T. carmanicus* (Lamiaceae) and *C. cyminum* (Apiaceae) due to phenolic compounds showed the highest antioxidant activity. Generally, the essential oils from some Iranian herbs can be used as an alternative preservative instead of synthetic ones in food industry. The results of this study reveal that some of species play an important role in primary healthcare system of these tribal communities.

**Key words:** Herb, GC/MS, monoterpenes, DPPH assay, different, healthcare

## INTRODUCTION

Due to concern about the safety of synthetic compounds, numerous bioactive secondary metabolites of plants are being used in various industries including pharmaceutical, chemical, cosmetic and food production (Gourine *et al*., 2010). The products of plant secondary metabolism such as essential oils, aromatics and volatile constituents have a wide range of applications in folk medicine, food flavoring and food preservation as well as in food industries (Pirbalouti, 2010), leading to increased use of natural substances and encouraging more detailed studies on the plant materials being used. Plant extracts with antioxidant and antibacterial activities could be promising agents in the food and flavoring industry (Imelouane *et al*., 2009; Buckingham, 1994). Secondary metabolites of herbs have been used in many domains, including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, smoking and other industrial purposes (Lubbe and Verpoorte, 2011).

The aerial parts of some species belonging to the family Lamiaceae including *Satureja bachtiarica*, *Satureja khuzestanica*, *Satureja hotensis*, *Thymus daenensis*, *Thymus carmanicus*, *Ziziphora clinopodioides*, *Ziziphora teniuor* and *Mentha longifolia* and the aerial parts of *Echinophora cinerea*, *Echinophora platyloba, Zaravschanica membranacea* and *Ferulago angulata* and the fruits (seeds) of *Heracleum lasiopetalum, Kelussia odoratissima* and *Cuminum cyminum* belonging to the family Apiaceae have been utilized as traditional medicines for antiseptic and antimicrobial effects as well as culinary and spice in Iran (Memarzadeh *et al*., 2015; Samani *et al*., 2015; Pirbalouti *et al*., 2016; Pirbalouti and Gholipour, 2016). The objectives of the present study were to evaluate the antioxidant activity of essential oils from medicinal and aromatic plants and to determine chemical compositions of essential oil from species of 15 Iranian medicinal herbs.

## MATERIALS AND METHODS

**Collection of plant samples:** Samples of Iranian medicinal and aromatic plants belonging to the families

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Lamiaceae and Apiaceae including the aerial parts of *Satureja bachtiarica*, *Satureja khuzestanica*, *Satureja hotensis*, *Thymus daenensis*, *Thymus carmanicus*, *Ziziphora clinopodioides*, *Ziziphora teniuor* and *Mentha longifolia* (Lamiaceae) and the aerial parts of *Echinophora cinerea*, *Echinophora platyloba*, *Zaravschanica membranacea* and *Ferulago angulata* (Apiaceae) and the fruits (seeds) of *Heracleum lasiopetalum*, *Kelussia odoratissima* and *Cuminum cyminum* (Apiaceae) were collected from wild populations of the plants growing in various alpine regions of southwestern Iran were used in this study (Fig. 1). Each sample was labeled and the location was recorded using a Global Positioning System (GPS, Vista Garmin) receiver.

Immediately following collection, the leaves, flowers and fruits from the plant samples were separated and bagged independently. The tissue samples were subsequently air-dried for 5-12 days in a shaded room at 30±5°C and then ground to a fine powder using a Moulinex food processor (Moulinex International, Spain). The ground, powdered samples were subsequently passed through a 20 mesh sieve to remove large pieces of debris in preparation for essential oil extraction.

**Essential oil extraction:** The essential oil of each sample was extracted from 50-100 g of powdered tissue by distillation, using a clevenger-type apparatus and following the procedures outlined in the British Pharmacopoeia. The sample was placed in a 2 L flask containing 1 L of water that was heated to 100°C for 3 h with a heating jacket to vaporize the oil that was subsequently condensed and collected. Essential oil samples were dried over anhydrous sodium sulfate and stored at 4°C until analyzed for constituents.

**Identification of the oil constituents:** Composition of the essential oils was determined by Gas Chromatography (GC) and Mass Spectrophotometry (GC/MS). The GC analysis was done on an Agilent Technologies 7890 GC (Agilent Technologies, Santa Clara, CA) equipped with a single injector and a Flame Ionization Detector (FID). A polar HP Innowax column and an apolar HP-5 capillary column (30 m×0.25 mm, 0.25 μm film thicknesses) coated with 5% phenyl, 95% methyl polysiloxane were used. The flow of the carrier gas ($N_2$) was 0.8 mL/min. Initial column temperature was 60°C and programmed to increase at 4°C/min to 280°C. The injector temperature was set at 280 and 300°C. Split injection was conducted with a ratio split of 1:40. Essential oil samples of 0.1 μL were injected neat (directly).

GC-MS analyses of aromatic oil samples were performed on an Agilent Technologies 7890 gas chromatograph coupled to Agilent 5975 C Mass Selective Detector (MSD) and quadrupole EI mass analyzer (Agilent Technologies, Palo Alto, CA, USA). A HP-5MS 5% column (coated with methyl silicone) (30 m×0.25 mm, 0.25 μm film thicknesses) was used as the stationary phase. Helium was used as the carrier gas at 0.8 mL/min flow rate. The temperature was programmed from 60-280°C at 4°C/min ramp rate. The injector and the GC-MS interface temperatures were maintained at 290 and 300°C, respectively. Mass spectra were recorded at 70 eV. Mass range was from m/z 50-550. The ion source and the detector temperatures were maintained at 250 and 150°C, respectively.

Oil constituents were identified based on their retention indices (determined with reference to homologous series of $C_5$-$C_{24}$ n-alkanes) by comparison of their mass spectra with those reported in the literature (Adams, 2007) and stored in NIST 08 (National Institute of


Fig. 1: The spice and condiments plants collected from the South Western Iran

Standards and Technology) and Willey (ChemStation data system) libraries. The peak area percentages were computed from HP-5 column without the use of FID response factors.

**Antioxidant test:** The DPPH radical scavenging activity of essential oils was determined using the method proposed by Huang *et al.* (2005). The essential oils (100 μL) at concentrations of 8, 16, 32, 62.5, 125, 250 and 500 μg/mL were mixed with 3.9 mL an equal volume of 0.2 mM ethanol solution of DPPH. The disappearance of the DPPH after 30 min of incubation at room temperature was determined using a Perkin-Elmer Lambda UV/Vis spectrophotometer at 515 nm against a blank, i.e., without DPPH. Ethanol was used to zero the spectrophotometer and the absorbance of the DPPH radical without antioxidant and measure daily served as the control. The amount of sample necessary to decrease the absorbance of DPPH by 50% ($IC_{50}$) was calculated graphically and the percentage inhibition was determined according to the equation:

$$\text{Inhibition (\%)} = \left[\frac{AC(0)\text{-}AA(t)}{AC(0)}\right]\times 100$$

Where:
AC(0) = The Absorbance of the control at t = 0 min and
AA(t) = The Absorbance of the antioxidant at t = 30 min

The food preservative Butylhydroxyanisole (BHA) was used as positive control. All measurements were replicated three times.

## RESULTS AND DISCUSSION

**Phytochemical analysis of essential oils:** Chemical compositions of the volatile oils from the aerial parts of *Satureja bachtiarica*, *Satureja khuzestanica*, *Satureja hotensis*, *Thymus daenensis*, *Thymus carmanicus*, *Ziziphora clinopodioides*, *Ziziphora teniuor* and *Mentha longifolia* (Lamiaceae) and the aerial parts of *Echinophora cinerea*, *Echinophora platyloba*, *Zaravschanica membranacea* and *Ferulago angulata* (Apiaceae) and the fruits (seeds) of *Heracleum lasiopetalum*, *Kelussia odoratissima* and *Cuminum cyminum* (Apiaceae) were analyzed by GC-FID and GC/MS.

According to results of GC-FID and GC/MS analysis, some chemical compounds identified in the essential oils from studied herbs, representing 89-99% of total oils which the main constituents of the herbs were monoterpenes oxygenated and hydrogenated monoterpenes. The main components of the volatile oils were γ-terpinene, thymol andcarvacrol in *S. bachtiarica*, *S. khuzestanica*, *S. hotensis*, *T. daenensis* and *T. carmanicus*; pulegone in *Z. clinopodioides*, *Z. teniuor* and *M. longifolia*; α-pinene in *Z. membranacea* and *F. angulata*; trans-β-ocimene in *E. platyloba*; α-phellandrene in *E. cinerea*; γ-terpinene and cumin aldehyde in *C. cyminum* (Fig. 2). Other identified chemical components in the essential oils from *H. lasiopetalum* and *Kelussia odoratissima* were n-octanol and trans-ligustilide, respectively.

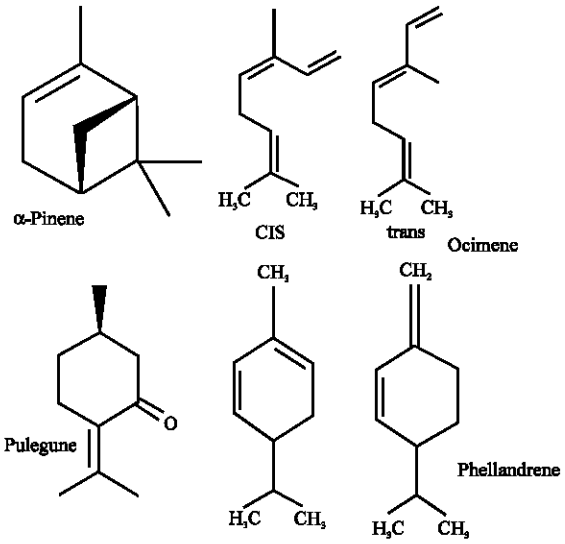


Fig. 2: The main constituents of the essential oils from the herbs

**Antioxidant test:** Antioxidant properties are very important in counteracting the deleterious role of free radicals in foods and biological systems. The potential antioxidant activity of the essential oils was determined by the scavenging activity of the stable free radical DPPH. Expressed as $IC_{50}$, the antioxidant activity for the essential oils extracted used in our study indicated the oil acted as an effective DPPH scavenger. The most antioxidant activity was exhibited by the essential oils from the aerial parts of *S. bachtiarica*, *S. khuzestanica*, *S. hortensis*, *Thymus daenensis* and *T. carmanicus* (Lamiaceae) and the fruits (seeds) *C. cyminum* (Apiaceae). Results of GC-FID and GC/MS analysis indicated that the main constituents of the studied herbs were monoterpenes oxygenated and hydrogenated monoterpenes in most species, expect *H. lasiopetalum* and *Kelussia odoratissima*. The results agree with previous reports that determined the major constituents of essential oils from the studied herbs collected in various provinces, Iran.

The DPPH is a stable free radical that in widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Fenglin *et al.*, 2004; Brand-Williams *et al.*, 1995; Pirbalouti *et al.*, 2013). Antioxidant molecules can quench DPPH radicals (by providing hydrogen atom or electron donation) and convert them to a colorless product

(Brand-Williams *et al*., 1995). The lower $IC_{50}$ value indicates a stronger ability of the extract to act as a DPPH scavenger while the higher $IC_{50}$ value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction. A main finding in this study was that the spice and condiment plants demonstrated much higher antioxidant activity and contained significantly more phenolic than other Iranian species of herbs that are considered as good natural sources of dietary antioxidants.

## CONCLUSION

Results of this study reveal that some of species play an important role in primary healthcare system of these tribal communities. Flora of the studied region appears to be a rich and interesting source for supplementary phytochemical studies. The Iranian herbs are ordinarily used for pharmaceutical purposes and also as health foods and spice in Iran. This study showed that the volatile oils from *S. bachtiarica*, *S. khuzestanica*, *S. hortensis*, *Thymus daenensis* and *T. carmanicus* (Lamiaceae) and *C. cyminum* (Apiaceae) possess a significant reducing free radical scavenging ability in vitro. In total, significant antioxidant activity of the essential oils of the studied herbs provide a scientific validation for the traditional use of the plant as an accessible source of natural antioxidants with consequent health benefits.

## RECOMMENDATIONS

Further, research on isolation and identification of active compounds and their efficacy need to be done. In addition, the antioxidative properties *in vivo* of studied herbs should be the objective of future research.

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