Original Article

Comparative Chemical Evaluation of Commercially Available Mint Hydrosols Produced in Fars Province, Iran

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Abstract

Background: Hydrosols of *Mentha* species are a common beverage among Iranians. The product quality may differ depending on the type of material used and the distillation process. However, despite the many investigations on the chemical composition of different mint essential oils, the co-produced hydrosols have rarely been evaluated. **Objectives:** This study evaluated and compared the chemical composition of 10 mint hydrosol samples purchased from local markets of Fars province, Iran, to *Mentha piperita* and *Mentha spicata* authentic hydrosols. **Materials and Methods:** Essential oils of the samples were extracted via liquid–liquid extraction by petroleum ether and analyzed using gas chromatography–flame ionisation detector (GC-FID) and gas chromatography–mass spectrometry (GC-MS). Hydrosols were then clustered based on their components. **Results and Conclusion:** Approximately 91.3%–100% of the components and overall 31 constituents were identified with the majority of oxygenated monoterpenes. Menthol, (R)-(−)-carvone, and piperitenone were the three different major compounds in the market samples. High percentages of menthol and carvone in many samples suggest that each product could be biologically active, and moreover, could be a valuable water-soluble source of their constituents. However, lack of chemical evaluation and standardization in this industry leads to inconsistency in quality and undermines the credibility of the industry.

Keywords: *Chemical composition, hydrosol, liquid–liquid extraction, Mentha*

Key message: *Although Iran is a pioneer in producing aromatic waters, the quality control of these products is still unsatisfactory by means of chemical composition. Reaching to a set of key standards will lead to greater value of these products.*

Introduction

The many biological activities of aromatic plants have a close relation to the chemical composition of their volatile oils.[1] Distillation is the most frequent method practiced for extracting the essential oil of many fragrant plants. During distillation, proportions of the water soluble components of the essential oil enter the water phase, producing hydrosols with a pleasant aroma. Unique biological and organoleptic properties of hydrosols have granted them much credit in a wide range of industries such as food, flavoring, cooking, cosmetic, perfumery, aromatherapy, and medicine.[2] Compounds passing into the aqueous phase are mainly oxygenated terpenes. They are responsible for the herbal scent and organoleptic properties[3] and have greater biological activity compared to the remaining hydrocarbon terpenes.[4] They are milder than essential oils and do not cause skin irritation and headaches.[5]

Aromatic waters are widely consumed as a beverage among Iranians. During recent years, they are extensively produced by many traditional and industrial companies and are finding their way in the market as natural and cheap pharmacological, flavoring, or cosmetic ingredients. Mint hydrosols are one of the best-selling hydrosol products all around the country. Genus *Mentha* (Lamiaceae) is best known for its aroma and essential oils[6] and has long been consumed to treat various diseases such as irritable bowel syndrome, flatulence, jaundice, diarrhea, catarrh, and nasal congestion in colds.

Genus *Mentha* is represented by seven species in Iran,[7] and the aromatic properties of its many species are highly dependent on various factors such as ecotype, climate and geographical conditions, and cultivation practices.[8] In addition to the variety of the raw material, different distillation processes may also affect the hydrosol product. Screenings have revealed that even the time of collection

How to cite this article: Nazemosadat Arsanjani Z, Etemadfard H, Moein M. Comparative chemical evaluation of commercially available mint hydrosols produced in Fars province, Iran. J Rep Pharm Sci 2020;9:52-8.

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Received: 16 Feb 2019
Accepted: 31 Aug 2019
Published: 26 Jun 2020

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Access this article online

Website: www.jrpsjournal.com

DOI:10.4103/jrptps.JRPTPS_17_19

Quick Response Code:

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during distillation may affect the odor and chemical composition of the hydrosol.[5] Counterfeit synthetic or semisynthetic products found in the market should also be considered. Adulteration is not uncommon for costly hydrosols. Man-made low value products by adding different natural/synthetic essences or chemical components to water or fortifying a natural hydrosol by adding desired chemicals are made by unauthorized producers.

It is clear that standardization of these products will help to ensure their quality, potential applications, and safety. However, unfortunately, there is neither legal definition nor grade or standards for hydrosols by industry, scientific community, international standard organization (ISO), and ANFOR (Association French Normalization Organization Regulation).[9] Documentation and evaluation of the hydrosols by chemical means could be a major step toward authentic products and honoring extra credit to this industry. The objective of this study was to analyze and compare the chemical composition diversity of 10 different mint hydrosols from local markets of Fars province, Iran, and two authentic *Mentha piperita* and *M. spicata* samples.

Materials and Methods

Hydrosol and plant material

Ten different brands of mint hydrosol were randomly purchased from local markets of Fars province, Iran, and numbered from M_1 to M_{10}.

To prepare *M. piperita* and *M. spicata* authorized hydrosols, aerial parts of the plants were harvested from the experimental garden of Faculty of Pharmacy, Shiraz, Iran, in November 2015. The specimen vouchers (*M. piperita*, MPPRC-95-1 and *M. spicata*, MPPRC-95-2) were kept at Medicinal Plant Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Extraction of *Mentha piperita* and *Mentha spicata* hydrosol

To assemble field distillation without cohabitation, 50 mg of the air-dried and powdered plant material was subjected to a full glass hydro-distillation apparatus for 3 h. The distillates were directly diverted to a collection flask so that essential oil and hydrosol were obtained simultaneously. The hydrosol was then separated from the essential oil by a separation funnel.

Essential oil recovering and concentration

To obtain the essential oil fraction of the hydrosol samples, liquid–liquid extraction technique was performed. Using a liquid extractor, 500 mL of hydrosols were recovered in 400 mL petroleum ether as an organic solvent at 40°C. For better extraction, after 4 h, the remains of the hydrosol were replaced by fresh sample and the process was repeated. The organic phase was then concentrated by a rotary evaporator (Heidolph Instruments, Schwabach, Germany) in 30°C under very low vacuum pressure to the volume of 10 mL. Remaining traces of water in the organic layer were dried over anhydrous sodium sulfate (Merck, Darmstadt, Germany). Each concentrated sample was kept in amber glass vials and in a cool place until use.

Gas chromatography–mass spectrometry analysis

Analysis of all samples was performed using gas chromatography–flame ionisation detector (GC-FID) Agilent Technologies, model 7890A, Santa Clara, USA apparatus attached to HP-5 ((5%-Phenyl)-methylpolysiloxane) column (30 m × 0.32 mm I.D., 0.25 µm film thickness). Nitrogen was used as carrier gas (flow rate: 1 mL/min, split ratio: 1:30). The injector and detector temperatures were adjusted at 250°C and 280°C, respectively. Column temperature was linearly programmed from 60°C to 250°C (at rate of 5°/min) and was held at 250°C for 10 min. Essential oil samples in petroleum ether (approximately 1%) were consecutively injected to the system.

By using the same method, constituents of the samples were identified via gas chromatography–mass spectrometry (GC-MS) (Agilent Technologies 7890 gas chromatograph coupled with an Agilent Technologies model 5975C mass detector). The apparatus was equipped with an HP5-MS capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness). The carrier gas was selected as helium with a flow rate of 1 mL/min. Mass spectrometer operated in electron ionization mode at 70 eV, and the mass scan ranged from 30 to 600 m/z. Kovats retention index was calculated for individual compounds using retention times of the reference *n*-alkanes (C7-C20). Identification of constituents was based on their calculated Kovats retention indices compared to Adams libraries and also based on the comparison of their mass spectra with those of authentic samples or with data already available in the literature.

Statistical analysis

To cluster hydrosols of different companies based on their components, hierarchical cluster analysis (HCA) and principal component analysis (PCA) methods were carried out using Minitab 17 software (Minitab, State College, Pennsylvania).

Results and Discussion

The essential oils of 10 commercially available mint hydrosols and authenticated *M. piperita* and *M. spicata* hydrosols were analyzed by GC/MS [Figures 1–3]. The simultaneous use of mass spectra and retention (Kovats) index matching led to the identification of 31 components representing 91.3%–100% of the total oil content [Table 1]. As expected, oxygenated monoterpenes were the main class of the chemical structures. HCA and PCA [Figures 4 and 5] between different samples indicated three subgroups, each of which was characterized by a principal compound; (R)-(−)-Carvone, menthol, and piperitenone. It should be noted that the results of each individual compound were limited by its diffusion coefficient and solubility in the organic phase and evaporation rate under the very low vacuum.[10]

As shown in Table 1, the amount of carvone ranged from 38.95% to 61.94% in the carvone subgroup (M_1, M_2, M_3, M_6, M_9, and

M. spicata). Carvone derivatives such as carvomenthone, neo-dihydrocarveol, dihydrocarvone, and carveol were also detected in high amounts in this subsection. Menthol was the major compound of *M. piperita*, M_7, and M_{10} (67.9%, 49.07%, and 31.05%, respectively). However, M_{10} by moderate amounts of carvone (23%) was distinguished from *M. piperita* and M_7 [Table 1] [Figure 4]. High portions of menthone were also found in these samples. In the third subcategory, the piperitenone content with a small variation was 41.73%, 42.4%, and 42.61%, in samples M_4, M_5, and M_8, respectively. Sample M_4 contains noticeable amounts of thymol (23%), which suggest contamination with plants such as *Thymus* spp.[11] or *Zataria multiflora* Boiss.[12]

Each crop contains different types and amounts of soluble fragrances. The major constituents and their quantity determine the product quality. (R)-(−)-Carvone is the most abundant compound in the volatile oil obtained from a number of mint species, particularly, spearmint oil[13] with a minimum of 51%,[14] and menthol is recorded to make up 29%–48% of peppermint essential oil.[15] They are widely used in food and perfumery industries due to their flavoring and fragrance properties, and also in pharmaceutical products used for colic, sour throat, and local pains.[14,16] Piperitenone and piperitone are common compounds in essential oils of *Mentha* spp., however, in low amounts. There are rare cases, which have reported piperitenone as a major constituent,[17] although piperitenone oxide is reported to be the main constituent of *M. longifolia* essential oil in some cases.[18,19]

In many parts of the world, hydrosols are often going to the drains as waste in the distillation process and their analyses are the subject of a limited number of publications.[20] The economic value of the hydrophilic essential oil fractions of aromatic oils that escape into the hydrosols was estimated to be worth US$50–US$100 million in India.[21] In this article, high amounts of menthol (67%) and (R)-(−)-carvone (52.2%) were observed in the authentic *M. piperita* and *M. spicata* hydrosols,

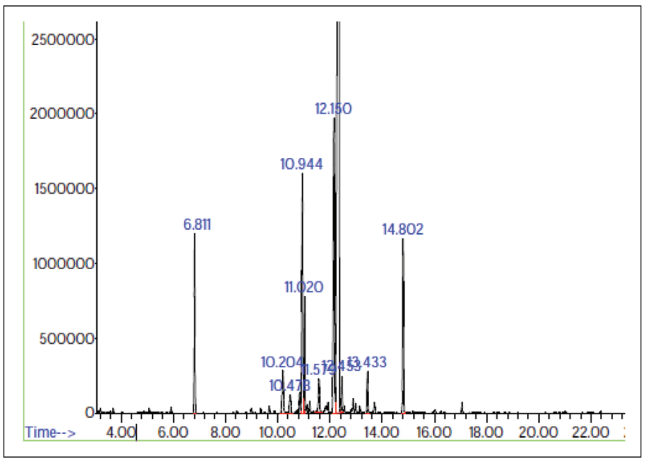

Figure 1: The gas chromatogram of M_1 hydrosol

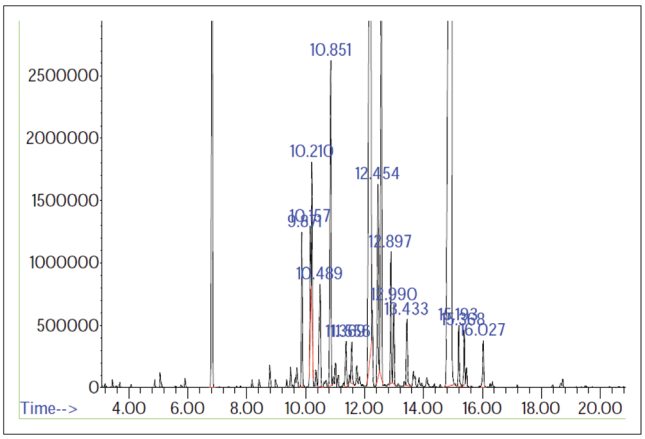

Figure 3: The gas chromatogram of M_8

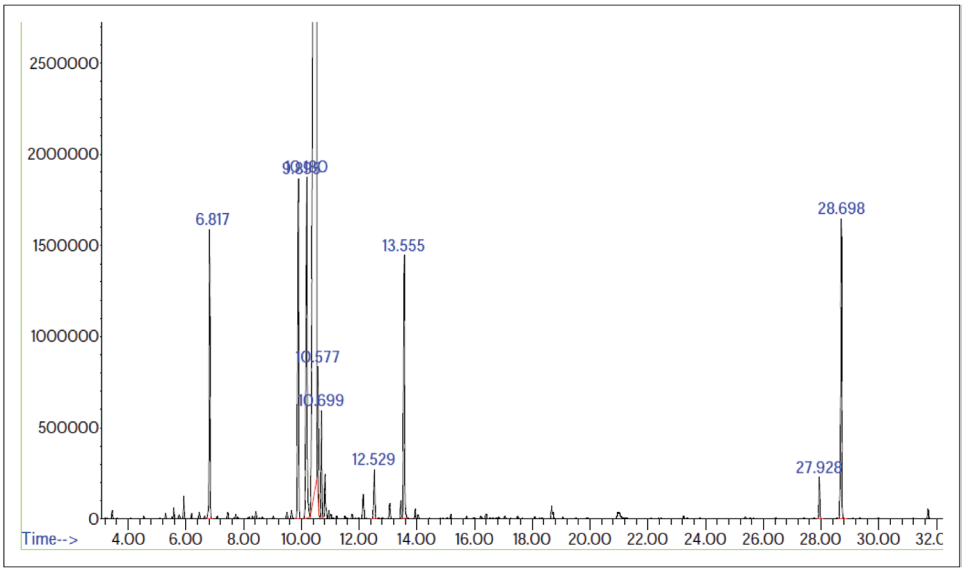

Figure 2: The gas chromatogram of *Mentha piperita*

Table 1: Composition (%) of the identified compounds in 10 mint hydrosol samples

Compounds	Sample										*M. piperita*	*M. spicata*
	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9	M_{10}		
p-Cymene	-	-	-	0.56		-	-	-	-	-		
Eucalyptol	4.46	7.13	8.02	0.9	5.11	6.97	4.44	10.29	8.83	7.27	5.05	2.67
γ-Terpinene	-	-	-	0.86	-	-	-	-	-	-		
Linalool	-	-	-	-	-	-	0.52	-	-	0.51		
Menthone	-	0.71	-	-	-	-	14.73	1.79	-	8.36	6.96	0.58
Isomenthone	-	0.71	-	-	-	-	7.73	1	-	1.07		
Neomenthol											8.77	
Borneol	1.95	1.26	1.97	1.31	1.96	-	-	1.65	0.98	-		2.09
Menthol	-	-	-	-	-	-	49.07	-	-	31.05	67.9	3.38
4-Terpineol	-	-	-	0.7	1.84	1.31	3.74	1.9	1.31	3.64	2.01	
Neoisomenthol							0.57				1.86	
Isopulegone	0.92	1.19	1.21	-	-	-	-	-	-	-		1.58
α-Terpineol	-	-	-	1.34	1.6	-	0.54	4.49	-	0.89		
Neo-dihydrocarveol	7.85	15.82	15.18	-	0.95	-		-	5.29	4.7		12.95
Trans-Isolimonene	-	-	-	-	-	4.22			-	-		
Trans-p-menthan-2-one	3.26	2.98	4.44	-	-	-	-	-	1.52	-		
Dihydrocarvone	-	0.43	0.64	-	-	2.02	-	-	-	2.98		1.45
Trans-carveol	1.43	0.95	0.76	-	-	1.6	-	-	1.43	0.37		
Cis-carveol	-	0.47	-	-	-	-	-	-	-	-		0.62
Pulegone	10.61	17.52	12.71	8.39	20.53	9.73	2.35	19.98	10.2	4.16		17.5
(R)-(−)-Carvone	61.94	38.95	46.63	1.63	6.16	61.22	2.84	-	60.68	23.62		52.2
Unknown	1		0.6	1.11	3.22	-	-	-	-	-		
Unknown	-	-	-	1.5	-	-	-	-	0.72	0.41		
Piperitone	-	0.4	-	-	0.91	-	2.04	6.79	-	1.59	1.12	
Thymol	-	1.06	3.38	23.13	0.97	-	7.13	-	-	2.48		
Neomenthyl acetate											5.5	0.66
Carvacrol	1.28	-	-	7.49	0.6	1.16	2.27	-	1.84	0.76		
Piperitenone	5.3	8.44	3.83	41.73	42.4	10.07	1.11	42.61	7.19	1.86		2.25
Eugenol	-	-	-	-	-	-	-	0.8	-	-		
Piperitenone oxide	-	-	0.63	8.5	1.35	-	-	-	-	-		
Unknown	-	-	-	-	3.92	-	-	-	-	-		
Unknown	-	-	-	0.83	2.48	-	-	-	-	-		
Unknown	-	-	-	-	-	-	0.49	-	-	-		
Methyl hexadecanoate											0.83	0.9
Unknown												0.64
Total	100	98.02	100	99.98	94	98.3	99.57	91.3	99.99	95.72	100	99.47

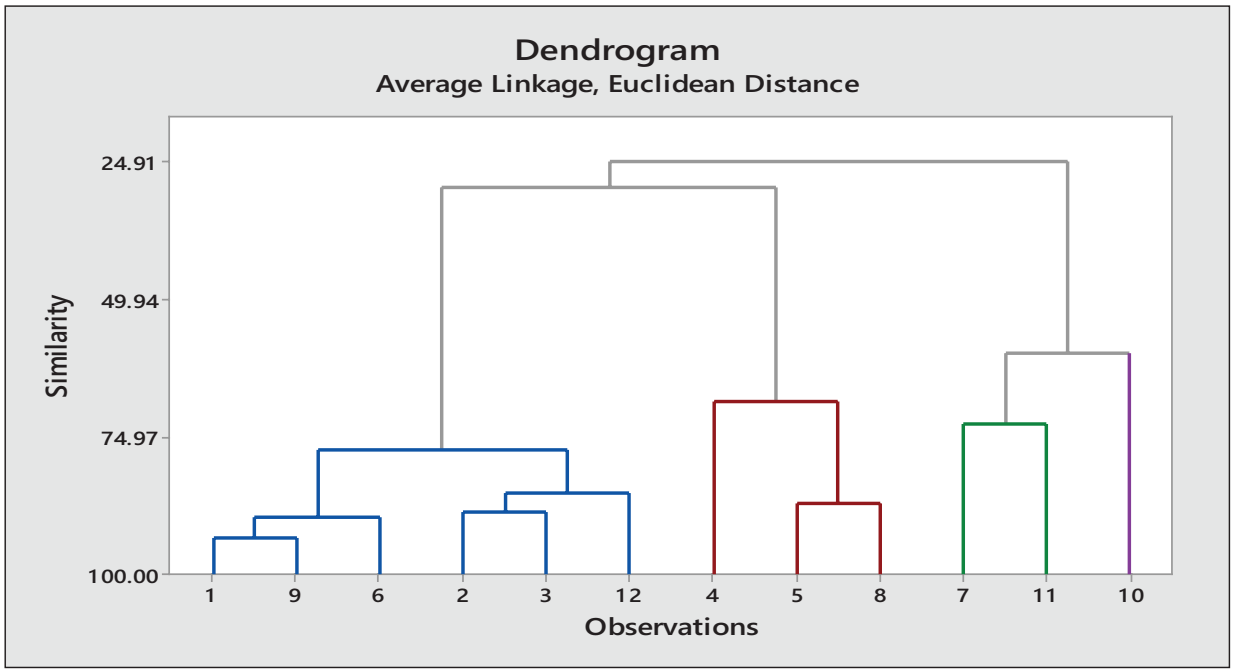

Figure 4: Dendrogram obtained by hierarchical cluster analysis based on the Euclidean distance between groups of essential oils of 10 mint hydrosols. Components that characterize the major subgroups are indicated

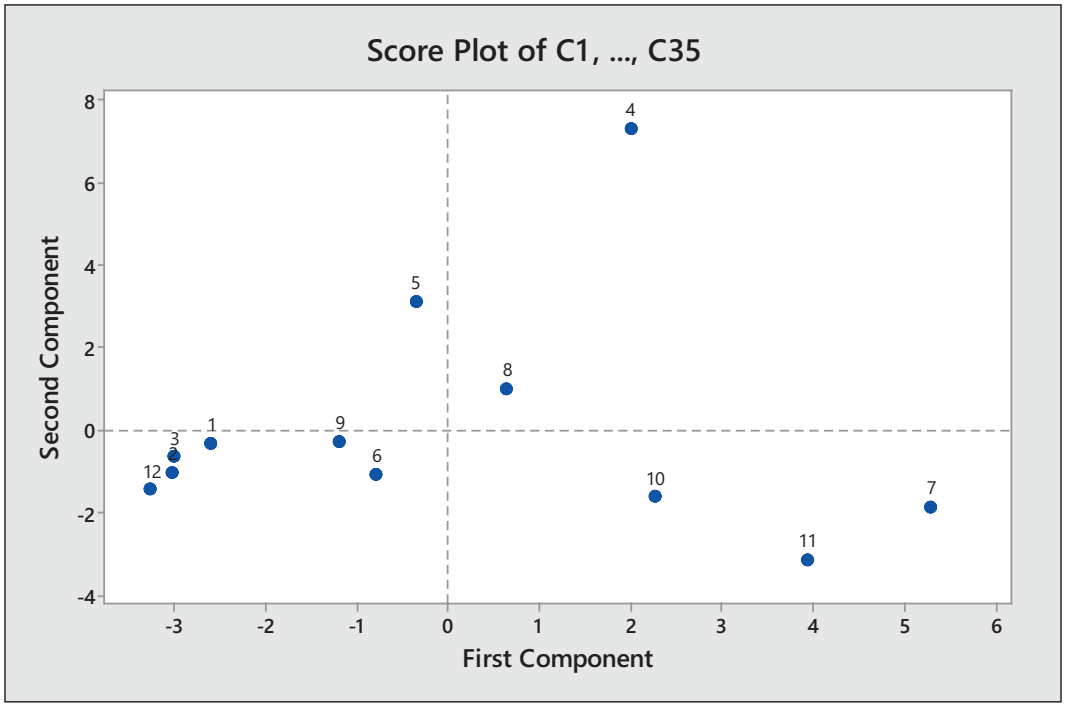

Figure 5: PC1/PC2 score plot by principal component analysis on the calibration set

respectively. This indicates that these side products could be a beneficial economic source for valuable compounds such as menthol and carvone.

Today with the emerging trend of using mild food preservation based on natural sources, aqueous phase hydrosols are good alternatives to nonpolar volatile oils where they have limitation of use. Different mint essential oils have shown good antibacterial effects[22] and are fungicidal against the plant pathogen (*Fusarium sporotrichioides*) responsible for damaging crops.[23] Testing inhibitory effects of different

hydrosols against *Salmonella typhimurium* and *Escherichia coli* O157:H7 inoculated into apple and carrots have shown that plant hydrosols can be used as a convenient sanitizing agent during the washing of fresh-cut fruits and vegetables.[24] Hydrosols are also used as mild pharmaceutical products for infants' colic relief. Moreover, nowadays aromatherapy practitioners are using hydrosols as mild bioactive ingredients in complementary alternative medicinal modality.[25]

Iran with its rich variety of aromatic plants is a pioneer in producing essential oils and hydrosols. Although to this day, unfortunately, it has not been able to achieve its global status and take full advantage of the industry. One of the main drawbacks is that neither the chemical composition nor the concentration of hydrosols' major soluble constituents is determined. Like any other industry, standardization is a crucial factor when hydrosols are going to be used for a specific pharmacological or biological purpose. For instance, the effectiveness of hydrosols as natural antimicrobials depends on the major soluble aromatics and whether or not they are in the range of the minimum inhibitory concentration/minimum lethal concentration of the microbe. Application of hydrosols in pharmaceutical or skin care products requires evaluated hydrosols to ensure that the amount of the soluble aromatics is enough for showing desired effects, and on the contrary, not high enough to cause adverse effects.

Conclusion

The results of this research have made it clear that there is a variation of chemical composition among mint hydrosols due to using different raw materials or distillation processes. This inconsistency in the products indicates lack of sufficient quality control by means of chemical composition. It is absolutely clear that developing relevant standards by the Iranian National Standards Organization for aromatic waters will help to ensure the consistency, safety, and effectiveness of the products. There is no doubt that high quality standardized products would have an important impact on the use of all potentials of the hydrosol industry and will lead to a more thriving industry.

Financial support and sponsorship

This study was financially supported by Shiraz University of Medical Sciences, Shiraz, Iran, grant number 93-01-70-7631.

Conflicts of interest

There are no conflicts of interest.

References

1. Naghibi F, Mosaddegh M, Mohammadi Motamed M, Ghorbani A. Labiatae family in folk medicine in Iran: From ethnobotany to pharmacology. Iran J Pharm Res 2005;4:63-79.
2. Andola H, Purohit V, Chauhan R, Arunachalam K. Standardize quality standards for aromatic hydrosols. Med Plants Int J Phytomed Relat Ind 2014;6:161-2.
3. Rao BR, Kaul P, Syamasundar K, Ramesh S. Chemical profiles of primary and secondary essential oils of palmarosa (*Cymbopogon martinii* (Roxb.) Wats. var. *motia* Burk.). Ind Crops Prod 2005;21:121-7.
4. Ait-Ouazzou A, Cherrat L, Espina L, Lorán S, Rota C, Pagán R. The antimicrobial activity of hydrophobic essential oil constituents acting alone or in combined processes of food preservation. Innov Food Sci Emerg Technol 2011;12:320-9.
5. Rao BR. Hydrosols and water-soluble essential oils of aromatic plants: Future economic products. Indian Perfum 2012;56:29-33.
6. Mozaffarian V. A Dictionary of Iranian Plant Names. Tehran, Iran: Farhang Moaser; 1996. Persian.
7. Jamzad Z. Lamiaceae. In: Flora of Iran. Vol. 76. Tehran, Iran: Research Institute of Forests and Rangelands; 2012. Persian.
8. Adelpoor MJ, Golparvar AR. Chemical composition of essential oils of three ecotypes of *Mentha spicata* L. from Kohgiluyeh va Boyer-Ahmad Province, Iran. J Herbal Drugs 2013;4:143-6.
9. Tannous P, Juliani R, Wang M, Simon J. Water balance in hydrosol production via steam distillation: Case study using lavandin (*Lavandula* x *intermedia*). In: New Use Agricultural and Natural Plant Products and ASNAPP Program. New Jersey, USA: The State University of New Jersey; 2004.
10. Garneau F-X, Collin G, Gagnon H, Pichette A. Chemical composition of the hydrosol and the essential oil of three different species of the Pinaceae family: *Picea glauca* (Moench) Voss., *Picea mariana* (Mill.) BSP, and *Abies balsamea* (L.) Mill. J Essent Oil Bear Plants 2012;15:227-36.
11. Nickavar B, Mojab F, Dolat-Abadi R. Analysis of the essential oils of two *Thymus* species from Iran. Food Chem 2005;90:609-11.
12. Saei-Dehkordi SS, Tajik H, Moradi M, Khalighi-Sigaroodi F. Chemical composition of essential oils in *Zataria multiflora* Boiss. From different parts of Iran and their radical scavenging and antimicrobial activity. Food Chem Toxicol 2010;48:1562-7.
13. Leitereg TJ, Guadagni DG, Harris J, Mon TR, Teranishi R. Chemical and sensory data supporting the difference between the odors of the enantiomeric carvones. J Agric Food Chem 1971;19:785-7.
14. de Sousa DP, de Almeida RN. Influence of the chirality of (R)-(-)- and (S)-(+)-carvone in the central nervous system: A comparative study. Chirality 2007;19:264-8.
15. Khan IA, Abourashed EA. Leung's Encyclopedia of Common Natural Ingredients: Used in Food, Drugs and Cosmetics. 3rd ed. Hoboken, New Jersey: John Wiley & Sons; 2011.
16. Kamatou GP, Vermaak I, Viljoen AM, Lawrence BM. Menthol: A simple monoterpene with remarkable biological properties. Phytochemistry 2013;96:15-25.
17. Mahboubi M, Haghi G. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. J Ethnopharmacol 2008;119:325-7.
18. Golparvar AR, Hadipanah A, Gheisari MM. Chemical analysis and identification of the components of two ecotypes of (*Mentha Longifolia* L.) in Iran province. Int J Agric Crop Sci 2013;5:1946.
19. Saeidi Z, Saeidi KA, Salehi A, Jouneghani RS, Amirshekari H, Taghipour A. Essential oil content and composition of *Mentha longifolia* (L.) Hudson grown wild in Iran. J Med Plant Res 2012;6:4522-5.
20. Garneau F-X, Collin G, Gagnon H. Chemical composition and stability of the hydrosols obtained during essential oil production. II. The case of *Picea glauca* (Moench) Voss., *Solidago puberula* Nutt., and *Mentha piperita* L. Am J Essent Oils Nat Prod 2014;2:29-35.
21. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants. Trieste, Italy:

United Nations Industrial Development Organization and International Center for Science and High Technology; 2008.

22. 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;6:147-53.
23. Rachitha P, Krupashree K, Jayashree GV, Gopalan N, Khanum F. Growth inhibition and morphological alteration of *Fusarium sporotrichioides* by *Mentha piperita* essential oil. Pharmacognosy Res 2017;9:74-9.
24. Tornuk F, Cankurt H, Ozturk I, Sagdic O, Bayram O, Yetim H. Efficacy of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli* O157:H7 and *Salmonella typhimurium* on fresh cut carrots and apples. Int J Food Microbiol 2011;148:30-5.
25. Price L, Price S. Understanding Hydrolats: The Specific Hydrosols for Aromatherapy: A Guide for Health Professionals. London, Churchill Livingstone; 2004.