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HPTLC, IR fingerprinting, and chemical composition analysis of commercial bitter orange (*Citrus aurantium* L.) hydrosols

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Abstract

Citrus aurantium L. hydrosol extracted by steam distillation from its flowers is a highly consumed herbal product in the Iranian traditional market, widely used as a food flavour and therapeutic food and drinks. This study investigated ten commercial hydrosol samples of *C. aurantium* flowers produced by conventional industrial processes, as well as a laboratory-prepared control sample. A liquid-liquid extraction method and sonication were used to extract essential oils from commercial hydrosols. Samples were then subjected to GC/MS analysis. ATR-IR spectroscopy was another efficient tool used to analyze hydrosol samples. All HPTLC chromatograms exhibited a close resemblance between samples and controls. The cluster analysis was used to compare the results of GC/MS and IR screening. In the HCA dendrograms derived from the GC/MS data, most of the oil sample profiles were found to be very similar to those of the control. Linalool was the most abundant compound in eight samples and the control. α-Terpineol in all hydrosol samples and geraniol in control and five other samples were the marker compounds. Ethyl disulfide, dillapiol, and hotrienol were detected in two samples that have not been reported in previous studies and might have resulted from the addition of other herbal hydrosols. Microbial content and pH values were within permissible limits in all samples, making them safe for oral consumption.

Keywords: *Citrus aurantium,* GC/MS, HCA, IR.

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1. Introduction

The Citrus family comprises 155 genera and over 1600 species containing various groups of bioactive secondary metabolites (1). Among the many species in this family, the blossoms and fruits of *Citrus aurantium* L., namely sour or bitter orange, are among the most widely consumed Citrus species (2). Various parts of this plant, including bark, flowers, leaves, fruits, and seeds, are used in folk and traditional Persian medicine for food and medicinal purposes, such as treating gastrointestinal diseases and nervous system disorders (3,4). The fruits and seeds of this plant have also been used as an anti-inflammatory, pain reliever, anthelmintic, and antidote to plant and animal poisons in traditional Persian medicine (5). Additionally, in modern medicine, various parts of the sour orange, including blossoms, have been revealed to exhibit antioxidant, weight-reducing, and antimutagenic properties (4, 6, 7).

The widely used blossoms of the bitter orange are consumed in fresh and dried forms in various food preparations, while bulk quantities of

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these blossoms are used for the production of the distillate called hydrosol.

Hydrosols are aqueous distillates consisting of herbal volatile constituents obtained by steam or hydrodistillation of various parts of plant species. They are considered secondary products in essential oil production and possess various culinary, cosmetic, and therapeutic properties (8). The bitter orange hydrosol is broadly consumed as a tonic and a traditional relaxing drink. So far, analysis of the volatile chemical composition of bitter orange blossoms has indicated the presence of linalool, linalyl acetate, limonene, pinene, geraniol, and nerol as the major bioactive components (9, 10). Many of these compounds have demonstrated antimicrobial, sedative, sleep-inducing, and anti-anxiety effects (11-13). *C. aurantium* flower hydrosol contains bioactive phenolic and terpenic compounds as the major chemical components contributing to the therapeutic properties. It is being prescribed by folk and traditional healers, in addition to their use as food and beverage ingredients (14, 15). Despite the abundant uses of *C. aurantium* flower hydrosol in food preparations and as a traditional food-medicine product, studies have yet to be conducted on further monitoring the quality of this plant product using instrumental methods of analysis to achieve proper effectiveness.

Based on the reports of many local producers, due to a limited bulk supply of sour orange flowers, to increase production volume and fulfil market demand, orange blossoms are mixed with those from tangerine (*Citrus reticulata*), as an adulteration, which obviously reduces the aroma quality and efficacy of these hydrosols.

Considering these adulterations and the widespread consumption of this hydrosol, the present study was designed to evaluate the chemical composition of commercial samples of bitter orange hydrosols offered in the traditional and industrial market of Fars province of Iran, using efficient techniques of instrumental analysis, including GC/MS, HPTLC, FTIR in addition to physicochemical characteristics.

2. Material and methods

2.1. Collection of market hydrosol samples

Ten market samples of orange hydrosols were prepared using two different methods, traditional and industrial, and were collected from authorized sellers. Table 1 shows the hydrosol, brand name, and the types of production methods used for each sample. As a control, a hydrosol sample was prepared from fresh sour orange flowers in the laboratory, using an extraction procedure almost identical to an industrial process used in the present study. A specimen of the sour orange flowers (control) used in this research was identified by the plant taxonomist, and a voucher (PM-852) was deposited in the herbarium of the School of Pharmacy, Shiraz University of Medical Sciences, before the laboratory extraction (Table 1).

2.2. Laboratory-prepared hydrosol as standard

Fifty grams of fresh, dried bitter orange blossoms were finely powdered in a laboratory grinder. Hydrosol was then extracted using hydrodistillation for 4 hours, similar to the distillation procedure performed in the usual industrial process and served as standard (STD). The resulting hydrosol was stored in a dark glass container at

Table 1. Specifications of *C. aurantium* flower hydrosols market samples.

Hydrosol Samples	Producer	Place of Origin
S1	Atragin	Meymand-Iran
S2	Naab	Meymand-Iran
S3	Aala	Meymand-Iran
S4	Golestan	Meymand-Iran
S5	GolAzin	Meymand-Iran
S6	Raeisi	Meymand-Iran
S7	Mohayyej	Meymand-Iran
S8	Khoshboo	Meymand-Iran
S9	Derakhshan	Meymand-Iran
S10	GolKooh	Meymand-Iran

4 °C until further analysis.

2.3. Separation of volatile components - Liquid-liquid extraction

Essential oils were extracted separately from each market hydrosol sample using a liquid-liquid extraction system. Each hydrosol sample (1 L) was extracted with n-hexane (2×500 ml) in two steps, and the organic layer containing volatile compounds was separated. The solvent was then removed at 45 °C on a rotary evaporator, and the essential oil was collected in dark glass tubes. The extraction process was repeated to remove the remaining volatile compounds of the hydrosol samples, and the essential oils obtained were stored at 4 °C until further analysis.

2.4. Volatile components of hydrosols – Sonication method

Each of the ten hydrosol samples and the control (7 mL) were transferred into separate tubes to extract the volatile components. n-Hexane was added to all tubes and placed in a sonicator for 20 minutes. The n-hexane layers bearing volatile organic components obtained from the triplicate extraction of each hydrosol sample were separated and pooled together. The prepared essential oil samples were dried over anhydrous sodium sulfate and subjected to a gentle stream of nitrogen. Each concentrated sample was then transferred into a separate screw cap test tube and stored at 4 °C before analysis.

2.5. Identification of volatile components - GC/MS

GC/MS was used to identify various chemical components of essential oil samples. This study used the Agilent GC instrument 7890 attached to a Mass spectrophotometer (MS) 5975C. The capillary column was HP-5MS (phenylmethyl siloxane 30mm×0.25 mm×0.25µm ID), Agilent Technologies Corporation. The initial column temperature was adjusted at 60 °C, while it reached 220 °C at 5 °C/min and remained at this temperature for 10 minutes. The helium was used as carrier gas at a flow rate of 1 mL/min. Mass spectra acquired at EI mode in a mass range of m/z 30-600. The voltage applied was 70eV, and the interfacial temperature was 280 °C (16).

2.6. GC/MS analysis of essential oil components

Each essential oil sample was diluted with n-hexane prior to injection into the GC spectrometer. A volume of 1 µL of the diluted essential oil solution was injected into the gas chromatograph in split mode with a split ratio of 1:50. The chemical components of the essential oils and control appeared in the form of individual peaks in the gas chromatogram, each with a specific retention time (RT) and the mass spectrum of each separated compound was recorded. Identification and quantification of essential oil components were initially performed by calculating KI (Kovats Index) values for each compound and then comparing the data with the information given in Wiley nl7, Adams (17), NIST, and Pherobase mass spectral libraries (18, 19).

2.7. High-performance thin layer chromatography (HPTLC) fingerprints

To prepare the TLC chromatogram, 10µl of each sample and the control methanolic solutions of essential oils were separately loaded on a silica gel plate 60 F254 (Merck) 10×20 cm. The loading condition of the samples was linear, with a bandwidth of 6 mm. A 10 ml volume of the mobile phase was used, and the length of its movement to the solvent front was set to 80 mm. The most suitable solvent system consisted of toluene-ethyl acetate (7.5:2.5 v/v). The plates were dried and examined under the visible and ultraviolet light of 254 and 366 nm wavelengths, and their photograph were recorded.

The developed and dried thin-layer chromatograms were further sprayed with sulfuric acid-anisaldehyde reagent and heated to 100 °C for ten min. to visualize the spots.

2.8. ATR-IR spectroscopy

IR spectroscopy was used to check the degree of similarity between the sample's chemical compositions. A fingerprint pattern was obtained for all samples. Bruker vertex-70 spectrometer and ATR apparatus were used to prepare the fingerprint. The range of IR spectrum for all samples was 756-12422 cm^{-1}. Data were entered into Mat Lab software for further analysis. The Standard Normal Variety (SNV) technique was used for the data matrix to eliminate baseline fluctuations (20).

2.9. Hierarchical Cluster Analysis

The ATR-IR spectroscopy data was analyzed using Mat Lab software (MathWorks Inc.) to draw Hierarchical Cluster Analysis (HCA) charts and analyze essential oil samples.

The essential oil composition percentage was considered a variable for this analysis, and data was created for each sample. The obtained matrix was entered into Mat Lab software to implement the HCA method. Cluster analysis using an unweighted pair group method (UPGMA) with Euclidean distance was used to measure the degree of similarity. A plot of data procured against samples was drawn to show the degree of similarity of the data (21).

2.10. pH measurement of essential oils

Laboratory pH measurements were conducted with a pH meter (Crison). In order to ensure accuracy, pH values were measured in triplicate using a calibrated pH meter, and average values were recorded.

3. Results

3.1. GC-MS analysis

GC/MS data of all the essential oil samples obtained from the bitter orange hydrosols was interpreted by comparing the profile of compounds in the MS chromatogram of each sample, which compared with that of the relevant n-alkane series and analyzed. The results are given in Table 2, which shows the types of compounds and the hy-

Table 2. Volatile composition of market samples of *C. aurantium* flower hydrosols.

	Bitter orange flower hydrosol samples (% of abundance)												
Constituent	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	STD	KICal.	KIRef.
Ethyl disulfide	-	-	-	-	-	-	-	-	0.49	0.36	-	925	925
Anisole	0.80	-	-	-	-	-	-	-	-	-	-	1021	923
Benzene acetaldehyde	-	-	-	-	-	-	-	-	-	-	6.19	1044	1044
Eucalyptol	-	-	-	-	-	-	-		-	0.46	-	1063	1053
Epoxy linalool	-	-	-	-	-	-	0.58	-	-	0.55	9.28	1073	1071
Linalool oxide	-	-	-	-	-	-	0.53	-	-	0.36	5.37	1089	1090
Linalool	65.11	63.56	70.34	66.10	-	64.55	45.65	68.36	42.45	12.21	33.99	1101	1101
Hotrienol	-	-	-	-	-	-	-	-	-	-	0.77	1105	1104
Phenetyl alcohol	-	2.53	-	2.82	11.75	-	5.49	-	2.24	3.52	0.61	1114	1114
Benzyl nitrile	-	-	-	-	-	-	-	-	-	-	1.06	1139	1140
4-Terpineol	-	-	-	-	-	-	1.02	-	-	-	-	1179	1180
α-Terpineol	10.41	12.81	15.64	14.14	-	13.96	15.89	20.69	12.7	3.54	13.37	1192	1193
α-Terpinolene	-	-	-	-	59.27	-	-	-	-	-	-	-	-
Dihydrocarveol	-	-	-	-	-	-	-	-	-	2.71	-	1196	1195
γ- Terpineol	-	-	-	-	-	-	0.52	-	-	-	-	1199	1199
γ-Terpinene	-	-	-	-	-	-	0.73	-	-	-	-	-	-
Dihydrocarvone	-	-	-	-	-	-	-	-	1.15	2.75	-	1200	1200
Isodihydrocarveol	-	-	-	-	-	-	-	-	-	0.55	-	1216	1214
Nerol	2.29	2.98	-	-	-	2.8	3.27	-	1.5	0.4	2.28	1229	1229
Homoveratrol	2.5	-	-	-	-	-	-	-	1.85	-	-	1230	1230
Pulegone	1.26	-	-	-	-	-	-	-	0.77	2.45	-	1242	1243
Carvone	0.84	-	-	-	-	-	-	-	-	-	-	1243	1246
Carvol	-	-	-	-	-	-	-	-	1.59	7.70	-	1246	1246
Anethole	-	-	-	1.91	-	-	-	-	-	-	-	1254	1253
Geraniol	5.46	5.84	-	-	-	5.48	16.00	-	3.24	-	6.54	1255	1255
p-Ethyl guaiacol	-	-	-	-	-	-	-	-	1.43	-	-	1280	1280
trans-Anethole	-	-	-	1.89	-	-	-	-	-	-	-	1287	1288
Thymol	2.99	-	-	-	-	-	0.90	-	4.22	4.56	-	1291	1292

Continued Table 2.

Indole	2.84	4.15	9.82	7.95	-	0.98	-	-	-	-	3.32	1294	1294
Carvacrol	1.12	-	-	-	9.98	-	0.52	-	9.26	1.56	-	1301	1301
p-Vinylguaiacol	0.95	-	-	-	-	2.70	-	-	-	-	1.56	1315	1315
Methyl anthranilate	-	4.68	-	-	-	4.30	-	-	1.72	-	13.12	1343	1342
Piperitenone	1.04	-	-	-	-	-	-	-	-	-	-	1347	1344
Eugenol	-	-	-	-	-	-	-	-	2.26	3.15	-	1359	1359
Isoeugenol	-	-	-	-	-	-	1.24	-	-	-	-	1360	1396
Myristicin	-	-	-	-	-	-	-	-	-	0.37	-	1524	1525
Nerolidol	0.72	1.84	-	-	-	0.94	-	-	-	-	-	1567	1565
Dillapiole	0.88	-	-	-	-	-	-	-	-	24.58	-	1629	1628
trans-Farnesol	0.79	-	-	-	-	0.92	-	-	-	-	1.19	1715	1722
Methyl palmitate	-	-	4.20	5.18	19.00	-	3.78	10.95	5.84	3.68	-	1916	1927
Methyl stearate	-	-	-	-	-	-	0.89	-	1.33	0.87	-	2126	2126
Identification	**100**	**98.39**	**100**	**99.99**	**100**	**96.63**	**97.01**	**100**	**93.55**	**75.97**	**98.65**	**-**	**-**

drosol samples listed in order. It represents the GC retention time for each specific compound and the calculated KI value for the identified compounds (Table 2).

The essential oil samples extracted from hydrosols are designated S1-S10, respectively, and the control was given S11. Figure 1 shows the proportion and the type of main components of the hydrosol samples having greater than 5% abundance (Figure 1).

3.2. HCA dendrogram based on GC/MS analysis

The HCA dendrogram derived from the GC/MS data is shown in Figure 2. All samples have been divided into three clusters. S1, S2, S3, S4, S6, S8, and S9 are similar to each other in terms of chemical components, and then S7 is close to them. These eight samples are all placed in the same cluster together with the standard sample. However, the next two clusters correspond to S10 and S5, each of which is placed separately in one cluster (Figure 2).

3.3. IR spectral analysis

The cumulative IR spectrum of all samples is presented in figure 3. As seen in the IR spectrum, the highest resemblance was observed among all samples and the control (Figure 3).

3.4. pH measurement

Table 3 shows the results of pH measurement in different samples of *C. aurantium* flower hydrosols. As mentioned, each sample was measured in triplicate, and the average value is reported in the last row. Sample S8 has the highest pH (7.35±.01), and sample S9 has the lowest pH

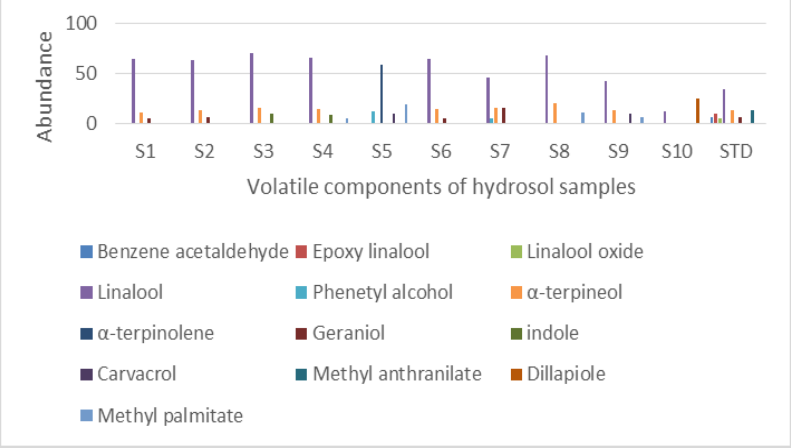

Figure 1. Major compounds of *C. aurantium* flowers hydrosol samples (abundance > 5%)

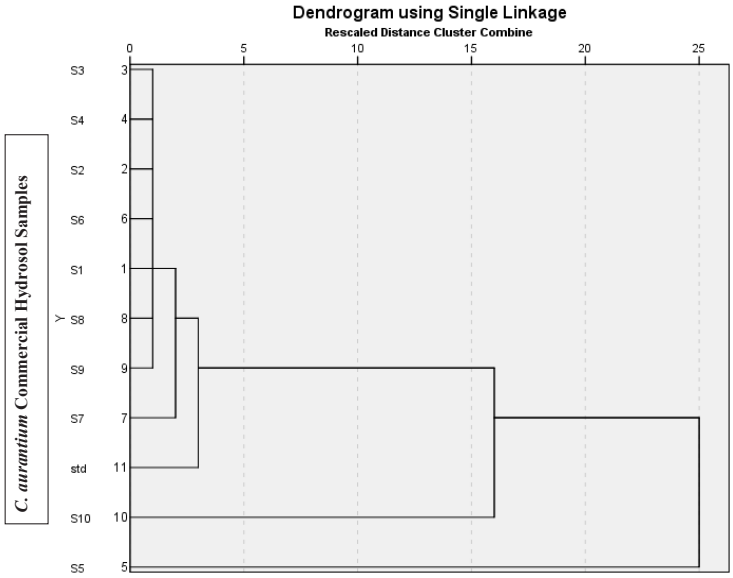

Figure 2. HCA Dendrogram from GC/MS data of all *C. aurantitum* flower hydrosol samples.

(5.42±.075) (Table 3).

3.5. HPTLC analysis

The HPTLC chromatogram of the volatile components of various hydrosol samples and control (standard) showing their relevant spots and resolutions is displayed in Figure 4.

4. Discussion

Plants have been the primary source of medicine since ancient times. Around 40–70% of people use herbal remedies as their first line of treatment for illnesses worldwide, and in developing countries, these plants play a crucial role in maintaining public health. Studies have shown that there are 40,000-50,000 species of plants in the world that are used through traditional and modern systems of medicine. The trade in medicinal plants has flourished perfectly due to this diversity (22).

The *C. aurantium* flower hydrosols contain a wide range of compounds, some minor in amount, but whose elimination significantly impacts their quality. Synthetic or low-quality samples of *C. aurantium* flower hydrosols lack these valuable compounds. However, some of these low-quality samples available in the market are adulterated. Considering this shortcoming, regular inspection and monitoring of the quality of *C. aurantium* hydrosols sold in the market is essential for public health concerns.

This study investigated the quality of 10 selected bitter orange flower hydrosol samples

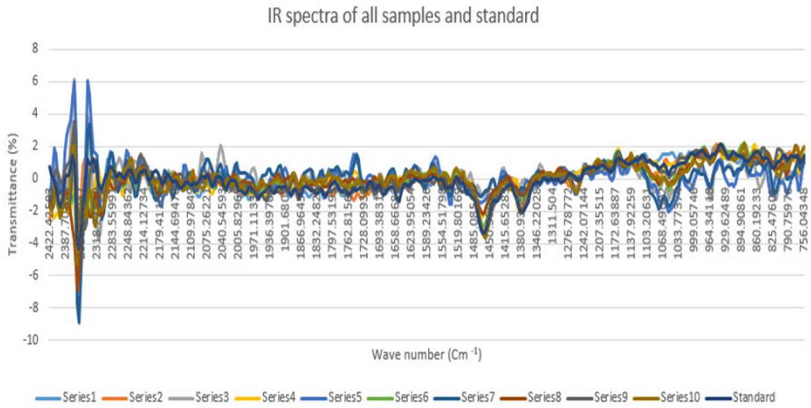

Figure 3. IR spectra of *C. aurantium* hydrosol samples and control.

Table 3. pH values of the tested hydrosol samples.

sample	*R1	R2	R3	Mean±SD
S1	6.43	6.98	6.80	6.74±0.28
S2	6.78	6.35	6.10	6.41±0.34
S3	6.30	6.15	6.21	6.22±0.07
S4	6.10	6.08	6.14	6.11±0.03
S5	6.98	6.94	6.92	6.95±0.03
S6	6.12	6.03	6.10	6.08±0.05
S7	6.70	6.68	6.65	6.68±0.03
S8	7.36	7.35	7.34	7.35±0.01
S9	5.50	5.42	5.35	5.42±0.08
S10	5.75	5.80	5.75	5.77±0.03
STD	6.68	6.67	6.59	6.65±0.05

*R= Replicate

from various producing companies in the Fars province market. This was achieved initially by extracting volatile components of each hydrosol sample, which was performed using the conventional hydrodistillation technique (23). However, this method has several disadvantages, such as the loss of several volatile compounds, the low yield, and the possibility of unsaturated compounds decomposing at high temperatures.

The constituents of volatile fractions of hydrosol samples were characterized using GC, an efficient analytical technique. To prepare HPTLC fingerprints, a small quantity of hydrosol samples was initially subjected to essential oil extraction using sonication (24, 25). However, this method is still in use due to its simplicity, lack of conventional organic solvents, and cost-effectiveness. The polarity profiles of each volatile component of hydrosol samples were successfully monitored using the thin-layer chromatograms prepared.

The essential oil of *C. aurantium* flowers contains monoterpenes, including linalool, which contributes directly to the plant's antioxidant properties (26). Linalool has a soothing effect on the central nervous system, including hypnotic, hypothermic, and anticonvulsant properties (27). Research has shown that linalool has local anaesthetic effects on the somatic sensory system (28). Inhalation of linalool-rich essential oils has reduced anxiety and aggressive behaviour and increased social interaction in rats (29). Combining linalool and the Shenque acupoint provided a more significant sleep regulation effect (30).

Furthermore, linalool has antimicrobial properties (31) and lethal effects on leishmania without causing cytotoxicity to mammalian cells (32). As shown in both *in vitro* and *in vivo* experiments, this compound inhibits inflammation, relieves tension, and improves menopausal symptoms (33, 34). Linalool has proven cytotoxic due to its induction and stimulation of cell death. It has great potential as a cancer treatment and immune booster (35). Based on Tables 1-3, aside from sample S5, all sour orange flower hydrosol samples contain linalool as the main compound. The highest linalool content was attributed to S3, with 34.70%.

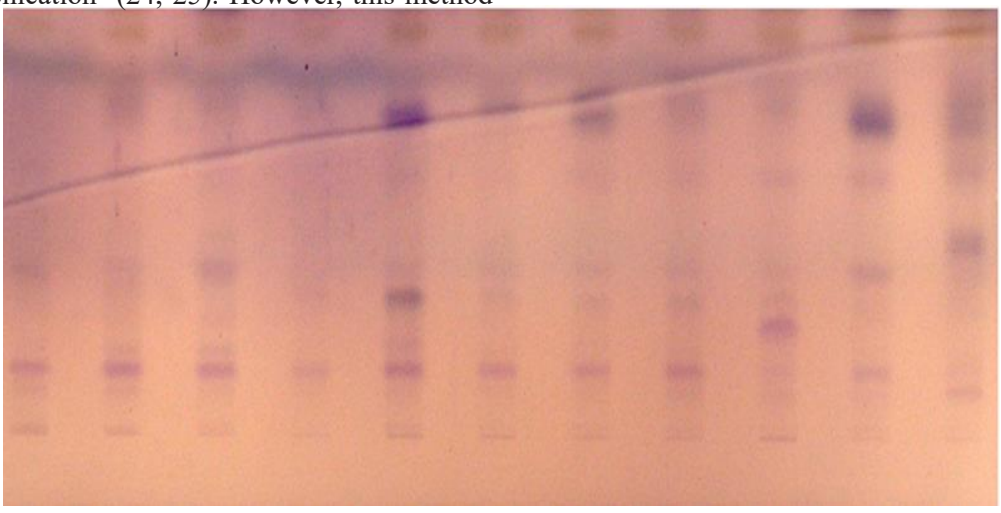

Figure 4. HPTLC chromatogram of hydrosol samples and control.

The hierarchical cluster analysis (HCA) is a clustering technique implemented to divide data based on similarities among hydrosol samples. The method is an efficient tool for classifying data quantitatively and is commonly used to compare the fingerprints of plant constituents (21).

GC/MS analysis indicated that except samples S5 and S10, the composition of rest of the samples were consistent with that of the standard (Table 2, Figure 1). Additionally, the dendrogram obtained using GC/MS data indicated that samples S5 and S10 had the least similarity to the standard sample. The primary purpose of conducting control tests to ensure the quality of herbal products is to identify and detect fraud. In this regard, essential oils and hydrosols used in food and medicine are prone to adulteration more than many other products. There is a possibility that some of the fake bitter orange hydrosols available on the market are produced by dissolving or mixing a minimal amount of orange blossom essential oil with synthetic linalool or linalool oxide in water, which may be considered fraudulent. Alternatively, a mixture of orange or mandarin synthetic essential oil in water may be presented on the market in place of bitter orange flower hydrosol.

According to the results of the GC analysis performed in this study, orange flower essential oil contained 94.5% limonene, 1% myrcene, 0.8% valencene, 0.7% linalool, and 0.3% each of octanal, decanal, and ethyl butyrate (36). The tangerine essential oil consists of 95.1% limonene, 2% myrcin, 0.5% α-pinene, β-pinene, linalool, and sabinene, and 0.2% octanal (37). While no hydrosol sample in this study contained a high content of limonene, which would be justified by the presence of tangerine or orange essential oil, one sample did not contain linalool, which might be construed as impure or fake. These products may also contain impurities like methanol, indicating another type of adulteration.

There is a possibility that unqualified processing and extraction of hydrosols from medicinal plants may result in undesirable components and may lead to health problems.

When plant organs with extra woody tissues undergo distillation, the resulting hydrosol contains high levels of methanol. This toxic substance is generated by the destructive distillation of wooden tissues. For this reason, plant materials must be devoid of extra wooden tissues before being used to extract herbal hydrosols through distillation. Methanol contamination of herbal hydrosols causes countless fatal poisonings. At the same time, long-term consumption of small amounts can also cause significant poisoning and blindness in frequent users of non-standard herbal hydrosols (38).

The presence of methanol in high concentrations in unhealthy hydrosols may result in liver damage and especially nerve cell poisoning. When consumed at low doses as a contaminant of non-standard herbal hydrosols in the long term, it may cause liver metabolic dysfunction, leading to inflammation and fatty liver disease (39, 40).

It is important to note that on a commercial scale, some of the hydrosols result from a double distillation process of plant materials with a higher concentration of chemical components and quality. To demonstrate the high quality of these hydrosols, the label on their packaging includes the word highly concentrated. In some cases, hydrosols are diluted with water, which has a very low concentration and quality. Hydrosols of this type are considered fraud by market inspectors and have lower levels of specificity and effectiveness.

It is critical to recognize that these hydrosols are composed of synthetic chemical compounds that may also be contaminated with unwanted chemicals that do not come from plants. Hydrosols containing these chemicals carry an artificial aroma that does not have the expected efficacy of herbal extracts. This could pose a health risk to humans.

However, the health problems that may arise from the consumption of synthetic hydrosol products may include headaches, allergies, and numerous skin complications (hives, pimples, spots, etc.) that may appear differently in individual people.

In the present study, in addition to evaluating essential oil compounds using gas chromatography, a thin-layer chromatography fingerprint was performed using the HPTLC technique to monitor a thin-layer pattern of the hydrosol components. Despite being convenient, this technique allowed

comparing the profile pattern of the major compounds present in various commercial samples of hydrosols with a standard sample. In addition to convenience, it offers advantages such as accuracy and reproducibility over the manual thin-layer methods.

Sonication was a simple, fast, and efficient laboratory extraction method for prompt investigation of plant products, particularly those relevant to quality assessment tests. This study mainly focused on the characterization of essential chemical components in commercial bitter orange flower hydrosol samples. It was performed with the aim of r chemical composition and, consequently, their health and expected therapeutic efficacy.

Based on the results of these instrumental methods, the unusual or harmful components of commercial hydrosol products could be efficiently detected. These tools contribute well to regular monitoring of these high-consuming plant products. In addition to simplicity and convenience, they are accurate and help achieve health and ensure the effectiveness of these food products. These experimental tools have great potential to be used as preventive tools, thereby conferring a comprehensive attenuating magnitude of various types of adulterations and minimizing the malnutrition that may be generated from this massive group of food products. However, additional in-depth studies and evaluations could help guarantee the health and efficacy of *C. aurantium* flower hydrosols presented in the market.

5. Conclusion

Given that plants are exposed to various contaminants, acceptable safety monitoring should be consistently performed to use the plant-derived products such as hydrosols as medicine or food to minimize the side effects of allergenic reactions and contaminants and provide a safe, reliable and standard product to the consumer. In the evaluation of the microbial contamination rate on commercial C. aurantium hydrosol samples, it was observed that in terms of microbial contamination, all hydrosol samples were within the permissible limit. Using GC/MS, the present study focused on the evaluation of the volatile chemical composition of C. aurantium hydrosol samples as a criterion to compare and evaluate their degree of conformity with standard and as a tool for monitoring their health. Other supplementary instrumental techniques such as HPTLC and IR were used to establish the proper quality control examination of the samples. HCA was used for the correlation assessment between the volatile composition of the samples from various producers and found helpful to categorize the market samples in various clusters in terms of similarity measures in chemical composition.

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Statistical analysis

Hierarchical cluster analysis (HCA) was used to group similar observations into a number of clusters based on the observed values of GC/MS. In HCA, Single-Linkage (nearest neighbour) was used as a similarity or distance criterion (41). This analysis was performed using SPSS statistical software version 16.0 (SPSS Inc., Chicago, IL, USA).

Conflict of Interest

The authors declare no conflict of interest.

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