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Volatile Compounds of Shade-Dried *Tussilago farfara* L. Using Purge and Trap Extraction Technique

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Abstract

The leaves and flowers of Coltsfoot (*Tussilago farfara* L.) have long been used for the therapeutic purposes, especially for respiratory ailments. Coltsfoot is also considered as a natural food flavoring due its aroma-rich property. In the present study, aroma compounds of shade-dried leaves and flowers of coltsfoot were isolated by purge and trap method and analyzed by GC and GC-MS. A total of 30 aroma compounds, including notably monoterpenes and sesquiterpenes were determined. Results showed that terpenes, such as; linalool (20.79 %), caryophyllene (16.36 %), *α*-pinene (9.72%), (*E*)-*β*-farnesene (9.07), germacrene (7.78%) and camphene (4.48%), were the most abundant compounds among overall aroma profile.

Keywords: *Tussilago farfara* L., Coltsfoot, purge and trap, aroma.

Öksürük Otunun (*Tussilago farfara* L.) Uçucu Bileşiklerinin Taşıyıcı ve Tuzak Yöntemiyle Belirlenmesi

Özet

Öksürük otunun yaprakları ve çiçekleri uzun yıllardır başta öksürük gibi boğaz yolu enfeksiyonları olmak üzere, birçok farklı hastalığa karşı tedavi amaçlı kullanılmaktadır. Ayrıca, aroma maddelerince zengin oluşu, öksürük otunun gıda sektöründe aroma verici olarak değerlendirilmesine olanak sağlamıştır. Bu çalışmada, gölgede kurutulmuş Öksürük otu yaprakları ve çiçeklerinin aroma maddeleri taşıyıcı ve tuzak yöntemiyle izole edilmiş, GC-MS cihazıyla analiz edilmiştir. Çalışmada elde edilen verilere göre, Öksürük otunda baskın olarak monoterpenler ve sesquiterpenlerin oluşturduğu toplamda 30 aroma bileşiği tespit edilmiştir. Bu bileşikler içerisinde Öksürük otunda linalool (% 20.79), karyofilen (%16.36), *α*-pinen (%9.72), (*E*)-*β*-farnesen (%9.07), germakren (%7.78) ve kamfen (%4.48) olduğu belirlenmiştir.

Anahtar kelimeler: *Tussilago farfara* L., Öksürük otu, purge and trap, aroma.

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Introduction

Tussilago farfara L. (coltsfoot), a member of *Asteraceae* plant family, is one of the remarkable perennial medicinal & aromatic plant widely distributed along Northern Hemisphere. Flowers and flower buds of this salubrious plant has long been used as a remedy of several respiratory ailments, especially in asthma and bronchitis, and also its leaves are consumed as a vegetable in Far-Eastern countries as well (Xu et al., 2017). A number of studies performed to deepen the knowledge about its phytochemical and pharmacological properties. Particularly, many of these works focused on the isolation of sesquiterpenoids, due to its salubrious effects, and qualitative analysis by means of several variations of chromatographic techniques (Kikuchi and Suzuki, 1992: Wang et al., 2011: Li et al., 2013: Song et al., 2017). Apart from the sesquiterpenes, some of the studies were also performed on its phenolics (Liu et al., 2014: Chanaj-Kuzmarek et al., 2013), pyrrolizidine alkoloids (Smyrska-Wieleba et al., 2016) and chromones (Wu et al., 2008) contents. Furthermore, some researchers were interested in metabolomic fingerprinting of *Tussilago farfara* L. recently (Li et al., 2013: Xue et al., 2012). In addition to these researches, there exist some studies carried out to investigate its anti-inflammatory (Hwangbo et al., 2009), antioxidant (Chang-Tian et al., 2012), antimicrobial (Kokoska et al., 2002), antitubercular (Zhao et al., 2014), insecticidal effects of this precious herb as well. Among all of this accumulated knowledge about important constituents and properties of *Tussilago farfara*, an extensive survey on aroma profile of this herb is still required.

Another important aspect for such medicinal and aromatic plants is the odour quality related to aroma compounds which play a key role to attract consumer's preferences. Many of MAPs (medicinal and aromatic plants) are being utilized as flavoring for food and beverages. *Tussilago farfara* is also listed as a natural food flavoring agent by the Council of Europe (Ferrer et al., 2016). Although, some of the studies have focused on the aroma profile of essential oil extracted from *Tussilago farfara* (Ferrer et al., 2016: Judzentiene and Budiene, 2011) aroma profile of shade dried flowers and flower buds of *Tussilago farfara* has not been deeply investigated yet. In earlier studies, it was declared that the aroma compounds of *Tussilago farfara* mainly constituted by terpenes especially monoterpenes and sesquiterpenes that are responsible for the citrus, herbal and floral notes.

Several aroma extraction techniques were utilized in order to perform better qualitative and quantitative surveys. As the massive number of constituents which have various polarities, volatilities are found in different concentrations and matrix. Therefore, the most critical steps of aroma analysis can be counted as the sample preparation, decision of correct extraction methodology and its optimization. The present study was organized to isolate aroma compounds of *Tussilago farfara* by purge and trap technique and investigate the total aroma profile by means of GC-MS system.

Materials and Methods

Samples and Chemicals

Commercial samples (1 kg) of dried *Tussilago farfara* L (origin: Turkey) were obtained from a local herbalist supplier, in Gaziantep, Turkey in July, 2016. The herbs were identified by the Faculty of Agriculture, University of Cukurova. The moisture content of the herb was 3.7-4.5% (dry basis). Water used in this study was purified by a Millipore-Q system (Millipore Corp., Saint-Quentin, France). The standard volatile compounds were purchased from Sigma-Aldrich (Steinheim, Germany). Dichloromethane, sodium sulfate and 4-nonanol were obtained from Merck (Darmstad, Germany). Dichloromethane was freshly distilled prior to use.

Extraction of Volatile Compounds

Volatiles of herb was extracted by purge and trap system which comprise a flow-meter that control the nitrogen source and connected to splitter system to divide the flow in several channels in order to purge three samples at the same time. Lichrolut EN tubes obtained from Merck was used as an adsorbent which is one of the most appropriate sorbents for volatile compounds extraction with respect to the previous research (Sonmezdag et al., 2017a). The herb samples

was previously mortared and placed into a 20 mL vial, then the sample was pre-incubated at optimized purging temperature (60 ^{0}C) for 10 minutes. The process was applied for 90 minutes with a nitrogen flow of 500 mL/min. After purging, the volatiles held in the cartridge were eluted with dichloromethane. The elute was dried by anhydrous sodium sulphate, the pooled organic extract was concentrated to 5 mL in a Kuderna Danish concentrator fitted with a Snyder column at 40ºC (Supelco, St Quentin, France) and then to 0.5 mL under a gentle flow of nitrogen. Extracts were then stored at -20°C in a glass vial equipped with a Teflon-lined cap until analysis. Extractions were carried out in triplicate.

Representativeness test for aromatic extract

Sample preparation and presentation. Tussilago farfara L. was evaluated using the descriptive and preference tests according to Poste et al. (1991). The panel was composed of ten assessors (seven females and three males between 20 and 45 years old) from Cukurova University, Food Engineering Department. The assessors were previously trained in sensory evaluation techniques. In the present study, we used a cardboard smelling strip (reference 7140 BPSI, Granger-Veyron, Lyas, France) for checking representativeness of the *Tussilago farfara* L. aromatic extract obtained with purge and trap extraction technique. Three grams of fined samples were placed in 15 mL brown coded flask as a reference for representativeness tests. Aromatic extract obtained from dichloromethane was adsorbed onto a cardboard smelling strip. After 1 min (the time necessary for solvent evaporation) the extremities of the strips were cut off, then placed in dark coded flasks (15 mL) and presented to the panel after 15 min. All the samples were assessed at room temperature (20 °C) in neutral conditions.

Similarity and intensity tests were performed to demonstrate the closeness between the odour of the extracts and the *Tussilago farfara* L. These test procedures were well documented in our previous study (Sonmezdag et al., 2016).

GC-FID, GC-MS Analysis of Volatile Compounds

Agilent 6890 chromatograph interfaced with flame ionization detector (FID) and Agilent 5973-Network-mass selective detector (MSD) (Wilmington, USA) constituted the gas chromatography (GC) system. DB-Wax column (30 m length x 0.25 mm i.d. x 0.5µm thickness, J&W Scientific Folsom, USA) was used to separate volatile compounds. 3µL of extract was injected in pulsed splitless (40 psi; 0.5 min) mode. Injector and FID detectors were set at 270°C and 280°C, respectively. The flow rate of carrier gas (helium) was 1.5 mL min^{-1}. The conditions of the oven program of the DB-Wax column was 50°C to 250°C at 4°C/min, 10 min hold. As for the mass-selective detector, the identical oven program was used. The MS (electronic impact ionization) conditions were as follows: ionization energy of 70 eV, mass range m/z of 30-300 a.m.u., scan rate of 2.0 scan s^{-1}, interface temperature of 250 °C, and source temperature of 180°C. The volatile compounds were analyzed in full scan mode and assigned by comparison of their retention index and their mass spectra on the DB-Wax column with those of a commercial spectra database (Wiley 6, NBS 75k) and the instrument's internal library made through the aforementioned laboratory researches. After identification, internal standard method with 4-nonanol was used to determine the mean value of volatile compounds and mean values (μg/kg dry weight; dw) of the triplicate of GC analyses were calculated for each sample. By using *n*-alkane (C_8–C_{32}) series, retention indices of the compounds were calculated (Sonmezdag et al., 2016).

Results and Discussion

An aromatic extract of T*ussilago farfara* obtained by purge and trap aroma extraction method displayed an identical aroma to the original odor of *Tussilago farfara*, when a drop of the aromatic extract was assessed on a cardboard smelling trip (7140 BPSI, Lyas, France). A certain amount of aromatic extract was injected into GC-MS system to perform a sensitive identification and quantification of each aroma compounds. Identified aroma compounds and their linear retention indices

determined on the DB-WAX column are given in Table 1.

According to results revealed in this study, several aroma compound groups were identified in the aromatic extract of *Tussilago farfara* herb including terpenes, alcohols, esters and a volatile phenol. In total, 30 compounds were identified in the overall aroma profile of *Tussilago farfara*. Terpenes were the most dominant aroma group constituting 94.25% of the overall aroma concentration. Many plants and parts of them are well known with their pleasant odors, spicy tastes or to show pharmacological activities due to the terpene compounds. These specialties are formed predominantly by terpenes. However, production purposes and biological functions of these compounds have not been completely inspected. Many herbs generate terpenes so as to charm insects for pollination or to protect herbs from being eaten by animals (Breitmaier et al., 2006). Among terpenes, linalool, caryophyllene, *α*-pinene, (*E*)-*β*-farnesene, germacrene and camphene were the five most abundant compounds and their concentrations were found as 37189µg/kg, 29276µg/kg, 17397 µg/kg, 16231 µg/kg and 14111 µg/kg, respectively. Similarly to *Tussilago farfara*, terpenes were also dominant compounds of MAPs such as *Salvia officinalis, Lavandula angustifolia,* and *Mentha asiatica* (Sonmezdag et al., 2017b). Terpene synthases are directly responsible for the production of these volatile terpenes. On the other hand, some of them are formed via modification of the main skeletons of terpene made by terpene synthases by hydroxylation, dehydrogenation, acylation, and other reactions (Dudareva et al., 2004). Number of investigations on volatile compounds of *Tussilago farfara* is very limited. Some studies on volatile oils only from buds of coltsfoot from Asia (as a Traditional Chinese Medical plant) have been reported. However, several new sesquiterpenoids and triterpenoids were isolated in coltsfoot inflorescence buds for the first time (Kikuchi et al., 2002; Yaoita et al., 1999).

Esters were the other important class of the aroma compounds in the *Tussilago farfara* L. Ester compounds have a very wide range of odor and flavoring effects and there are over 200 of these compounds permitted for use in foods. Moreover, these compounds are widely distributed in the essential oils and in some instances represent the major constituent. Generally, ester compounds are responsible for the mature and fruity notes (Reinneccius, 2005). Linalyl acetate and endobornyl acetate were the identified and quantified ester compounds in the herb. Linalyl acetate has significance impact compound in the perfume industry and is found in large amounts in various plants (Casabianca et al., 1998).

Another important chemical group of volatiles was alcohols. These compounds are formed from fatty acids supplied to excised tissue, by 8-oxidation followed by reduction in two step from acetyl-coenzyme A to aldehyde and aldehyde to alcohol (Knee and Hatfield; 1981). 3-penten-2-ol, 3-octanol, 1-octen-3-ol, and butoxyethoxy ethanol were the alcohol compounds with the concentration of 284 µg/kg, 1028 µg/kg, 567 µg/kg, 1637 µg/kg, and 2192 µg/kg, respectively.

Conclusions

In the present paper, the aim was to determinate the aroma compounds of *Tussilago farfara* L., a member of *Asteraceae* plant family, cultivated in Turkey. Thirthy aroma compounds were identified in herbs including, alcohols, esters, phenol and terpenes. Terpene compounds were determined as the main chemical group among the identified aroma compounds followed by alcohols. 23 terpene compounds were identified in the samples. Linalool, caryophyllene, and *α*-pinene were most important terpene compounds detected in all *Tussilago farfara* L.

Table 1. Volatile compounds of *Tussilago farfara* L.

No	LRI*	Compounds	Concentration(µg/kg) #	Identification§
1	1029	*α*-Pinene	17397	LRI,MS,std
2	1057	Camphene	8007	LRI,MS,std
3	1116	2-*β*-pinene	1268	LRI,MS,tent
4	1150	3-Penten-2-ol	284	LRI,MS,std
5	1161	*β*-Myrcene	7345	LRI,MS,std
6	1176	*α*-Terpinene	1473	LRI,MS,std
7	1142	*Δ*-3-Carene	1896	LRI,MS,tent
8	1204	*dl*-Limonene	5188	LRI,MS,std
9	1212	*β*-Phellandrene	2938	LRI,MS,tent
10	1273	*p*-Cymene	1611	LRI,MS,std
11	1396	3-Octanol	567	LRI,MS,std
12	1429	Acetic acid	120	LRI,MS,std
13	1456	1-Octen-3-ol	1637	LRI,MS,tent
14	1493	Camphor	1847	LRI,MS,std
15	1530	*β*-Bourbonene	1579	LRI,MS,tent
16	1547	Linalool	37189	LRI,MS,std
17	1555	Linalyl acetate	1386	LRI,MS,std
18	1558	Endobornyl acetate	1666	LRI,MS,tent
19	1594	4-Terpineol	807	LRI,MS,tent
20	1606	*β*-Gurjunene	3074	LRI,MS,tent
21	1625	Caryophyllene	29276	LRI,MS,std
22	1681	*α*-Amorphene	956	LRI,MS,tent
23	1690	Isoborneol	2919	LRI,MS,std
24	1711	(*E*)-*β*-farnesene	16231	LRI,MS,std
25	1716	Germacrene-d	14111	LRI,MS,tent
26	1752	*Δ*-Cadinene	2920	LRI,MS,std
27	1758	*γ*-Cadinene	5602	LRI,MS,tent
28	1827	Butoxyethoxy ethanol	2192	LRI,MS,tent
29	2005	Nerolidol	5959	LRI,MS,std
30	2222	Carvacrol	1462	LRI,MS,std
		Total	**178922**	

* LRI, linear retention index calculated on DB-WAX capillary column

Concentration: Results are the means of three repetitions as μg kg^{-1} dw. Standard deviation of all volatile compounds was <5 %.

§ Identification: Methods of identification; LRI (linear retention index), MS tent. (Tentatively identified by MS), Std (chemical standard); When only MS or LRI is available for the identification of a compounds, it must be considered as an attempt of identification. nd (not detected).

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