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The Volatile Compounds of the Elderflowers Extract and the Essential Oil

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Abstract: *Sambucus nigra* L. (Caprifoliaceae) known as 'black elder' is widely used as both food and medicinal plant in Europe. Elderflowers are consumed as herbal tea and its gargle has benefits in respiratory tract illnesses such as cough, influenza, inflammation in throat. In this study, we aimed to show the compositions of the volatile compounds-rich in extract and the essential oil of the elderflowers cultivated in Kütahya, Turkey. HS-SPME (Headspace-Solid Phase MicroExtraction) technique was employed to trap volatile compounds in the hexane extract of dried elderflowers. The volatile compounds in the essential oil from elderflowers isolated by hydrodistillation were analyzed GC and GC-MS systems, simultaneously. Results for the *n*-hexane extract: thirty volatile compounds were identified representing 84.4% of the sample. *cis*-Linalool oxide (27.3%) and 2-hexanone (10.5%) were found to be main compounds of the *n*-hexane extract. Results for the essential oil: fifteen volatile compounds were identified representing 90.4% of the oil. Heneicosane (18.8%), tricosane (17.3%), nonadecane (13%) and pentacosane (10.3%) were the major compounds of the oil.

Keywords: *Sambucus nigra*; elderflowers; HS-SPME; GC-MS; essential oil; volatiles.

1. Plant Source

Sambucus nigra was acquired from Çamlıca Province, Kütahya, Turkey. The flowers collected in June, 2014 were obtained from Hekim Sinan Medicinal Plants Research Center, Kütahya. The plant identification was done by Nazım Tanrıkulu (Hekim Sinan Medicinal Plants Research Center, Kütahya, Turkey). The voucher samples were avaliable at Hekim Sinan Medicinal Plants Research Center.

2. Previous Studies

In the literature, steam distillation, distillation-extraction based on the Likens-Nickerson principle, supercritical extraction, pentane extraction and dynamic headspace sampling reported by different research groups were employed for isolation and concentration of volatile compounds from elderflowers [1].

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Toulemendo and Richard [2] reported that dry elderflowers identified with containing 16 hydrocarbons, 11 ethers and oxides, 7 ketones, 7 aldehydes, 16 alcohols, 6 esters, and 16 acids. Among these groups, *trans*-3,7-dimethyl-1,3,7-octatrien-3-ol (13%), palmitic acid (11.3%), linalool (3.7%), *cis*-hexenol (2.5%), and *cis*- and *trans*-rose oxides (3.4% and 1.7%, respectively) were the main compounds in the essential oil obtained by steam distillation. They were also principal components of the isopentane extract and of the ethanol concentrate. All samples had a good muscat odor [2].

Jorgensen et al. [3] identified fifty nine compounds in the elder elderflower syrup made from fresh elderflowers by dynamic headspace technique and analyzed by GC and GC-MS. The odor of the volatile compounds was detected by GC-sniffing technique. This group reported that *cis*-rose oxide, nerol oxide, hotrienol, and nonanal contributed to the characteristic elder flower odor, whereas linalool, α-terpineol, 4-methyl-3-penten-2-one, and *(Z)-β*-ocimene contributed with floral notes. Fruity odors were associated with pentanal, heptanal, and *β*-damascenone. Fresh and grassy odors were primarily correlated with hexanal, hexanol, and (*Z*)-3-hexenol [3]. In 2008, Kaack recorded that fifty two aroma compounds including 9 aldehydes, 7 ketones, 22 alcohols, 3 esters, 4 oxides, 6 terpenes and 1 hydrocarbon in elder flower extracts obtained from cultivated samples [1]. In another study, volatile compounds emitted from elderflower tea samples were collected by dynamic headspace technique (purge and trap) and analyzed by GC and GC-MS. A total of fifty six volatile compounds were identified and quantified, including ten aldehydes, seven ketones, twenty one alcohols, one phenol, three esters, four heterocycles, and eight hydrocarbons being derivatives of fatty acids, amino acids, shikimic acid and/or of terpenoid origin [4].

3. Present Study

The dried elderflowers (53g) were macerated with *n*-hexane (1000, 700, 700 mL) by shaker for three times (8 hours). The hexane extract was dried under vacuum by rotary evaporator (<35°C). The extract was obtained by yielding 1.9% of the flowers. To trap volatile compounds in the *n*-hexane extract HS-SPME technique was employed. The manual SPME device (Supelco, Bellafonte, PA, USA) with a fiber precoated of a 65 µm thick layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB-blue) was used for extraction of the elderflowers volatiles. The vial containing the elderflowers *n*-hexane extract was sealed with parafilm. The fiber was pushed through the film layer for exposure to the headspace of the extract for 30 min at 50°C. The fiber was then inserted immediately into the injection port of the GC/MS for desorption of the adsorbed volatile compounds for analysis.

The essential oil from elderflowers was isolated by hydrodistillation for 3 h using a Clevenger-type apparatus. The pale yellow essential oil obtained by trace amount of the elderflowers was dissolved with *n*-hexane and stored at +4˚C until analyzed. The volatile compounds in the essential oil were analyzed GC and GC/MS systems, simultaneously.

The GC-MS *(Gas chromatography-Mass spectrometry) analyses;* were done with an Agilent 5975 GC-MSD system. An Innowax fused silica capillary (FSC) column (60 m × 0.25 mm, 0.25 µm film thickness) was used with helium as the carrier gas (0.8 mL/min). Oven temperature was kept at 60°C for 10 min, then programmed to 220°C at a rate of 4°C/min, then maintained constant at 220°C for 10 min, and finally programmed to 240°C at a rate of 1°C/min. Injector temperature was set at 250°C. Split flow was adjusted at 50:1. Mass spectra were recorded at 70 eV with the mass range *m/z* 35–450.

GC analyses; were performed using an Agilent 6890N GC system. FID (Flame Ionization Detector) detector temperature was set to 300°C and the same operational conditions were applied to a duplicate of the same column used in GC-MS analyses. Simultaneous auto injection was done to obtain equivalent retention times. Relative percentages of the separated compounds were calculated from integration of the peak areas in the GC-FID chromatograms.

Individual components were identified by computer matching with commercial mass spectral libraries (Wiley GC-MS Library, MassFinder 3 Library) and in-house "Baser Library of Essential Oil Constituents", which includes over 3200 authentic compounds with Mass Spectra and retention data from pure standard compounds and components of known oils as well as MS literature data, were also used for the identification [5-8]. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes [9].

Thirty volatile compounds including 3 ketones, 6 alcohols, 8 terpenoids, 3 aldehydes, 2 alkane hydrocarbons, 3 aromatic hydrocarbons, 4 carboxylic acids and 1 ester were identified representing 84.4% of the *n*-hexane extract. *cis*-Linalool oxide (pyranoid) (27.3%) and 2-hexanone (10.5%) were found to be main compounds of the extract. Fifteen volatile compounds containing 1 ketone, 3 terpenoids, 1 alkane hydrocarbon, 1 carboxylic acid, 7 alkanes, 1 ester and 1 aromatic compound were identified representing 90.4% of the essential oil. Heneicosane (18.8%), tricosane (17.3%), nonadecane (13%) and pentacosane (10.3%) were the major compounds of the oil. For the details (Table 1).

As seen, the *n*-hexane extract was rich in terpenoids (36.1%), ketones (17.0%) and alcohols (15.7%) while the essential oil was rich in alkanes (61.8%) and alkane hydrocarbons (13.0%). In the same time, aldehydes (6.1%), carboxylic acids (4.7%), aromatic hydrocarbons (2.2%), esters (1.6%) and alkane hydrocarbons (1.0%) were identified in the *n*-hexane extract. Terpenoids (6.0%), carboxylic acids (3.7%), ketones (3.2%), esters (1.4%) and aromatic volatiles were found in the essential oil. When compared the results, different volatile compounds except hotrienol and nonadecane were identified in both samples.

Farkas et al., [10] reported that 3-methylthio-1-propanol, ethyl-4-hydroxybutanoate, 2-phenylethanol, *cis*-rose oxide, *trans*-rose oxide, hotrienol, eugenol, and were found to be responsible for the characteristic flavor of macerates from fermented elder flowers [10]. Merica et al. [11] shared the GC-MS analysis of elder flowers evidenced the presence of 39 compounds, among which 13 hydrocarbons (C7-C36), 16 esters, palmitic acid, 9 compounds of various structures, containing oxygen such as α-amyrin, γ-sitosterol, linalyl oxide, vitamin E [11]. The volatile compounds of the aqueous solution of fresh elder flowers obtained by the dynamic headspace technique were analyzed by GC-FID and GC-MS. According to analyzes, 59 compounds were identified. The amounts of identified volatiles were measured in five elder cultivars and significant differences were detected among cultivars in the concentration levels of 48 compounds. The majority of the constituents were of terpenoid origin and included monoterpenes, irregular terpenes, and one sesquiterpene, *β*-caryophyllene. According to the GC-sniffing technique, *cis*-rose oxide, nerol oxide, hotrienol, and nonanal contributed to the characteristic elder flower odor, whereas linalool, α-terpineol, 4-methyl-3-penten-2-one, and (*Z*)-*β*-ocimene contributed with floral notes. Fruity odors were associated with pentanal, heptanal, and α-damascenone. Fresh and grassy odors were primarily correlated with hexanal, hexanol, and (*Z*)-3-hexenol [3].

4. Conclusions

The medicinal uses of elder elderflowers are known from ancient times. It has been used as diuretic, antidiabetic and for antiviral and immune-boosting effects, treating flu, common colds and fever for a long time. Elder elderflowers also are consumed as food such as syrup, tea and beverages. Especially in Europe, commercialization process has been developed for the traditionally prepared drinks. Because of the multipurpose uses, qualitative and/or quantitative analyzes of the chemical compositions of elderflowers are very important for their not only phenolic compositions but only aroma compounds. So, the flavor and aroma compounds of elderflowers are highlighted by researchers. Different techniques on isolation of the volatile compounds were developed and used. When compared our results, the headspace-SPME technique was more effective for the isolation of the volatiles from the flowers than the traditional hydro-distillation.

The headspace technique may play important role for the efficient separation, identification, qualification and quantification of volatile from elder flowers and their products.

When the published data summarized, elderflowers have a strong flowery and pleasant odor. Important contributors to the floral and elderflower flavour of the extracts were rose oxides, hotrienol, linalool, linalool derivatives and α-terpineol, whereas the fruitiness and freshness of the extracts were mainly due to non-oxidized monoterpenes, aliphatic aldehydes and alcohols [12]. The aroma composition of elderflowers includes aldehydes, ketones, alcohols, esters, oxides, terpenes, free fatty acids.

Our results have good related with published data with small differences. The reason for these differences in the literature, different elderberry cultivars grown under different environmental conditions have been investigated and that different extraction and concentration techniques have been used for the isolation of the volatile compounds.

Table 1. The volatile compounds of elderflower extract and the essential oil

RRI[a]	Compound	Type	Odor description*	A (%)[b]	B(%)[b]	Identification method
1058	3-Hexanone	Ketone	Sweet, musty	6.2	-	MS
1087	2-Hexanone	Ketone	Acetone like, pungent	10.5	-	MS
1202	3-Hexanol	Alcohol	Grassy	4.0	-	MS
1222	2-Hexanol	Alcohol	Green, bitter, almond, mushroom	6.2	-	MS
1362	*cis*-Rose oxide	Terpenoid	Flowery, rose	-	2.2	MS
1376	*trans*-Rose oxide	Terpenoid	Flowery, rose, elderflower	-	1.1	MS
1400	Nonanal	Aldehyde	Faint elderflower	0.8	-	MS
1400	Tetradecane	Alkane hydrocarbon	Green	0.3	-	t_R, MS
1446	2,6-Dimethyl-1,3(*E*),5(*Z*),7-octatetraene (*E*,*Z*-cosmene)	Terpenoid	Floral	0.6	-	MS
1460	2,6-Dimethyl-1,3(*E*),5(*E*),7-octatetraene (*E*,*E*- cosmene)	Terpenoid	Floral	0.9	-	MS
1450	*trans*-Linalool oxide (furanoid)	Terpenoid	Elderflowers, leaves, sweet	1.7	-	MS
1475	Acetic acid	Carboxylic acid	Vinegar	0.6	-	t_R, MS
1478	*cis*-Linalool oxide (furanoid)	Terpenoid	Flowery, sweet	0.4	-	MS
1541	Benzaldehyde	Aldehyde	Candy, sweet	1.4	-	t_R, MS
1553	Linalool	Terpenoid	Flowery, freesia	0.3	-	t_R, MS
1616	Hotrienol	Terpenoid	Floral, sweet	1.3	2.7	MS
1694	*p*-Vinylanisole (4-methoxystyrene)	Aromatic	Sweet	-	1.3	MS
1750	*cis*-Linalool oxide (pyranoid)	Terpenoid	Herbaceous, fresh	27.3	-	MS
1763	Naphthalene	Aromatic hydrocarbon	Strong, mothball	0.7	-	t_R, MS
1770	*trans*-Linalool oxide	Terpenoid	Floral	3.6	-	MS

	(pyranoid)					
1845	(*E*)-Anethol	Alcohol	Anise	1.4	-	t_R, MS
1870	Hexanoic acid	Carboxylic acid	Sweaty Cheese, rancid, fatty	2.1	-	t_R, MS
1884	1-Methylnaphthalene	Aromatic hydrocarbon	Mothball	1.2	-	MS
1896	Benzyl alcohol	Alcohol	Floral, rose, slightly sweet	1.7	-	t_R, MS
1900	Nonadecane	Alkane hydrocarbon		0.7	13.0	t_R, MS
1912	2-Methylnaphthalene	Aromatic hydrocarbon	Coal tar	0.3	-	MS
1937	Phenyl ethyl alcohol	Alcohol	Floral, rose	0.7	-	t_R, MS
1949	(*Z*)-3-Hexenyl nonanoate	Ester		1.6	-	MS
1965	2-Ethyl hexanoic acid	Carboxylic acid	Paint, vanish	1.7	-	MS
1981	Heptanoic acid	Carboxylic acid	Fatty, dry	0.3	-	MS
1984	2-Acetyl pyrrol	Ketone	Caramel, burnt	0.3	-	MS
2000	Eicosane	Alkane		-	3.7	t_R, MS
2053	Anisaldehyde	Aldehyde	Sweet, mimosa, hawthorn	3.9	-	t_R, MS
2100	Heneicosane	Alkane		-	18.8	t_R, MS
2131	Hexahydrofarnesyl acetone	Ketone	Wine-like	-	3.2	MS
2182	2-Phenoxyethanol	Alcohol	Flowery, fresh	1.7	-	t_R, MS
2200	Docosane	Alkane		-	3.5	t_R, MS
2240	1-Methyl ethyl hexadecanoate	Ester		-	1.4	MS
2300	Tricosane	Alkane		-	17.3	t_R, MS
2400	Tetracosane	Alkane		-	3.6	t_R, MS
2500	Pentacosane	Alkane		-	10.3	t_R, MS
2700	Heptacosane	Alkane		-	4.6	t_R, MS
2931	Hexadecanoic acid	Carboxylic acid	Plastic, green	-	3.7	t_R, MS
			TOTAL	84.4	90.4	

Identification method: t_R, identification based on the retention times (t_R) of genuine standard compounds on the HP Innowax column; MS, tentatively identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data. [a]RRI: Relative retention indices. [b]% calculated from FID relative peak area data. *, odor description from published data. (-) not identified. (A) elderflower *n*-hexane extract; (B) the essential oil.

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