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RESEARCH ARTICLE

Determination of essential oil component composition of common sunflower marginal flowers

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ABSTRACT:
Common sunflower is a valuable crop that is used widely in food industry. The whole plant has a strong aromatic odor. Macroscopic analysis of its raw materials reveals glandular structures, milk vessels, and conceptacles, which allows supposition on presence of volatile components. We have studied essential oil component composition of common sunflower leaves, calathidiums, seeds, and roots earlier. Taking into consideration the fact that folk medicine uses a tincture of common sunflower marginal flowers as an anti-inflammatory, appetizing, anesthetic, and antispasmodic agent, we studied qualitative composition and quantitative content of essential oil components of common sunflower marginal flowers for the first time. Essential oil was extracted by the method of hydrodistillation. Using chromatography-mass spectrometry, we determined 30 compounds, 20 of which were identified. The total content of volatile components in essential oil was 598.73 mg/100g. The following substances were among those identified: aromatic compound (para-cymen-8-ol), monocyclic (α-pinene, α-campholenic aldehyde, terpinene-4-ol, trans-carveol) and bicyclic monoterpenoids (verbenene, verbenol, verbenone, cis- and trans-sabinenehydrate, myrtenol, bornylacetate), acyclic triterpenoid (squalene), monocyclic (germacrene D, β-bisabolene), bicyclic (β-caryophyllene) and tricyclic (calarene) sesquiterpenoids and saturated hydrocarbons (pentacozane, geptacozane, nonacozane). It was verbenol from the group of bicyclic monoterpenoids (105.16 mg/100g), calarene from the group of tricyclic sesquiterpenoids (48.42 mg/100g), and germacren-D from the group of monocyclic sesquiterpenoids (42.06 mg/100g) that accumulated most. Further studies of chemical composition of common sunflower marginal flowers of and development of herbal drug products on their basis are promising.

KEYWORDS: annual sunflower, marginal flowers, essential oil, gas chromatography, hydrodistillation.

INTRODUCTION:
Common sunflower (*Helianthus annuus* L.*)*, being a valuable crop, is used in production of oil in food industry, compound animal feedstuff in agriculture, and biofuel in energetics. In folk medicine, they use a calathidium tincture, which has anti-inflammatory and anesthetic action in rheumatism and gout[1]. A tincture of marginal flowers is used for bronchial spasms, gastrointestinal cramps, neuropsychic disorders, and decreased appetite[2].

The whole plant has a strong aromatic odor. Macroscopic analysis of its raw materials reveals that flower epidermis is covered with glandular structures; in addition, there are milk vessels and conceptacles, which allows supposition on presence of essential oil in flowers[3]. We have studied essential oil component composition of leaves, calathidiums, including both, marginal and median flowers, envelope and common phoranthium, as well as seeds and roots[4,5,6,7,8]. Previously the chemical composition of organic and fatty acids of leaves and anthodiums, including marginal flowers, a involucre and a flat receptacle was studied. It is calarene, verbenol, squalene that dominate in common sunflower calathidiums, whereas germacrene D dominates in leaves, and spathulenol and β-bisabolene – in roots[4,5]. In aerial parts of plants the following terpenoids were found: helikauranoside A, grandifloriacid, paniculosid, and kaur-16-en-19-oic acid[6]. Spanish scientists found heliannone A, annuionone E, heliannuol L, helibisabonol A and B, heliannuol A and D, and leptocarpin in leaves[7,8]. We have found no information in literature on studies of essential oil extracted from marginal flowers, separated from calathidiums.

The aim of the work was to extract essential oil fractions from marginal flowers of a common sunflower and to determine volatile components of essential oil.

MATERIALS AND METHODS:

For study purposes, we took marginal flowers harvested during mass flowering period at scientific and research plots in Kharkiv region in 2016. Essential oil was obtained by the method of hydrodistillation[9]. Exact weight (0.5 g) of the material transferred into a 20-mL vial and added internal standard. As an internal standard, we used tridecane, in an amount of 50 µg per exact weight, with further calculation of internal standard concentration obtained, which was then used for calculations. We added 10 mL of water to the sample and removed volatile components of the sample with water vapor for 2 hours using air-cooled reflux. In the process of removal, volatile substances are absorbed on the internal surface of the reflux. Substances absorbed after system cooling were rinsed with slow adding of 3 mL of extra-pure grade pentane into a 10-mL dry vial. The rinse was concentrated by a flush (100 mL/min) of extra-pure grade nitrogen to extract residual volume of 10 µL, which was completely sampled by a chromatographic syringe. Further concentration of the sample was carried out in the syringe itself to the volume of 2 µL. The content of volatile components of essential oil was studied on a gas chromatograph Agilent Technology 6890 with a mass-spectrometer detector 5973. Sample injection (2 µ) into a chromatographic column was carried out in splitless mode, i.e. without split ratio, which allowed sampling without loss on splitting and improved significantly (10-20-fold) sensitivity of chromatography method. Sample injection rate was 1.2 mL/min, for 0.2 minutes. Chromatographic column: capillary INNOWAX, 30 m long, with internal diameter 0.25 mm. Carrier gas velocity (helium): 1.2 mL/min[10]. Injection temperature: +250˚ C. thermostat temperature was programmed from +50˚C to +250˚ C with velocity of 4 degrees/min. Mass-spectrum library NIST05 and WILEY 2007 with total number of spectra more than 470,000 in combination with programs for identification AMDIS and NIST were used for component identification. A method of internal standard was used for quantitative calculations.

Calculation of component content was carried out by the following formula:
C=K1*K2*1,000, where
K1=P1/P2 (P1 – peak area of the test substance, P2 – peak area of the standard).
K2=50/W (50 – weight of the internal standard (µg) introduced into the sample, W – sample weight (mg)).

RESULTS AND DISCUSSION:

In the result of the study, 30 compounds were determined in essential oil of common sunflower marginal flowers, 20 of which were identified (see Table 1, Fig. 1). The following substances were among those identified: aromatic compound (para-Cymen-8-ol), monocyclic (α-pinene, α- campholenic aldehyde, terpinene-4-ol, trans-carveol) and bicyclic (verbenene, verbenol, verbenone, cis- and trans-Sabinenehydrate, myrtenol, bornyl acetate) monoterpenoids, acyclic triterpenoid (squalene), monocyclic (germacrene D, β-bisabolene), bicyclic (β-Caryophyllene) and tricyclic (calarene) sesquiterpenoids and saturated hydrocarbons (pentacozane, geptacozane, nonacozane).

Table 1. Chemical composition of essential oil volatile components of common sunflower flowers.

Retention time. min	Name of essential oil components	Content. mg/100g	Retention time. min	Name of essential oil components	Content. mg/100g
7.61	α–Pinene	0.92	24.09	Germacren D	42.06
8.29	Verbenene	0.51	24.47	β-Bisabolene	3.51
12.49	trans-Sabinenehydrate	5.34	24.79	unknown substance	2.92
13.08	unknown substance	3.28	24.89	unknown substance	6.4
13.64	cis-Sabinenehydrate	11.48	26.57	unknown substance	6.69
14.44	α-Campholenic aldehyde	8.86	27.76	unknown substance	103.05
15.43	Verbenol	105.16	28.78	unknown substance	97.48
16.52	Terpinen-4-ol	17.81	34.55	unknown substance	5.68
16.97	p-Cymen-8-ol	6.66	34.90	unknown substance	13.91
17.23	Myrtenol	8.87	35.07	unknown substance	6.99
17.49	Verbenone	19.84	35.27	unknown substance	6.81
18.06	trans-Carveol	15.24	37.06	Pentacozane	5.19
19.63	Bornylacetat	4.48	38.99	Geptacozane	10.06
22.9	β-Caryophyllene	7.11	40.06	Squalene	15.78
23.17	Calarene	48.42	40.79	Nonacozane	8.22

The total content of essential oil volatile components in common sunflower marginal flowers was 598.73 mg/100g. Comparative analysis of compounds identified as for their chemical groups showed that it was bicyclic monoterpenoids that accumulated most (155.68 mg/100g). Among seven compounds of this group identified, verbenol was most abandoned (105.16 mg/100g). Tricyclic sesquiterpenoids are represented by calarene (48.42 mg/100g). The content of monocyclic sesquiterpenoids was 45.57 mg/100g, and the share of germacrene D was 42.06 mg/100g. The quantity of monocyclic monoterpenoids was somewhat less, 42.83 mg/100g, among which terpinene-4-ol accumulated most (17,81 mg/100g). The sum of saturated hydrocarbons was 23.47 mg/100g. Triterpenoid squalene was present in essential oil in the quantity of 15.78 mg/100g, and the content of bicyclic sesquiterpenoids and aromatic compounds was insignificant, 7.11 mg/100g and 6.66 mg/100g, respectively.

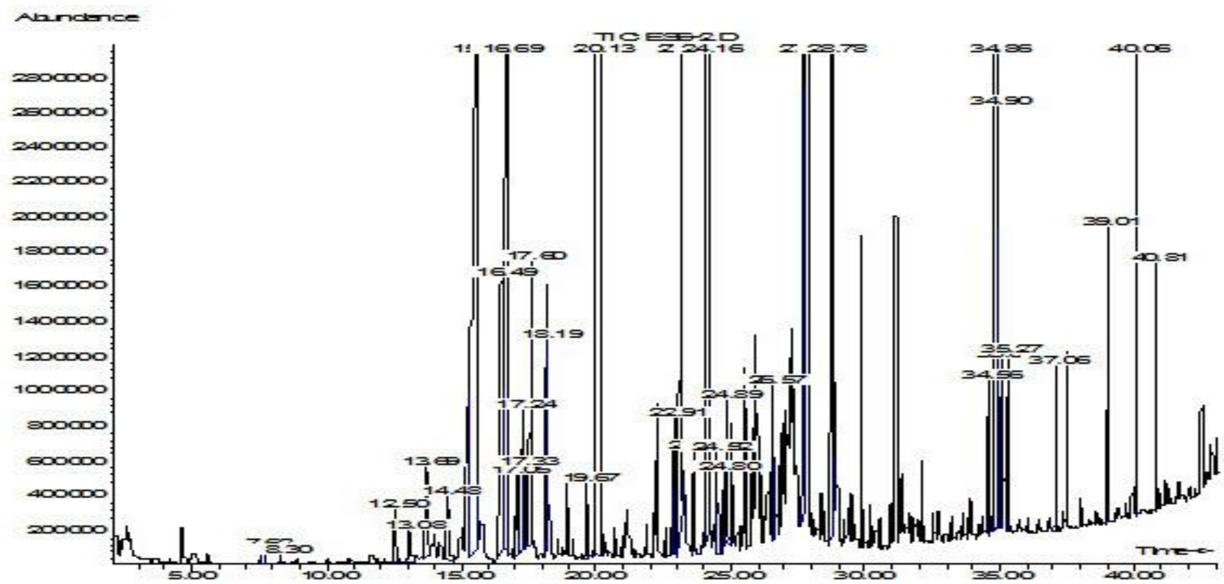

Fig. 1. Chromatographic profile of essential oil volatile components of common sunflower marginal flowers.

CONCLUSIONS.

It is the first time, when essential oil composition in common sunflower marginal flowers was studied using chromato-mass-spectrographic method.

In the result of the study conducted, 20 compounds were identified in raw materials. The following ones accumulated most: verbenol from the group of bicyclic monoterpenoids (105.16 mg/100g), calarene from the group of tricyclic sesquiterpenoids (48.42 mg/100g), and germacren D from the group of monocyclic sesquiterpenoids (42.06 mg/100g).

Thus, marginal flowers of common sunflower are promising raw materials for further study, extraction of biologically active compounds, and development of herbal drug products on their basis.

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