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CHEMICAL COMPONENTS IN THE ESSENTIAL OILS AND ULTRASONIC EXTRACTION OF AMANUS SAGE (*PHOLOMIS LONGIFOLIA* VAR. *LONGIFOLIA BOISS*. & BL)

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Abstract

In the present study the essential oil components of the flowers and leaves of Amanus sage (*Pholomis longifolia* var. *longifolia* Boiss. & Bl) by ultrasonic extraction and steam distillation method were determined. According to the chemical composition analysis of Amanus Sage plant using ultrasonic extraction method, major components were methyl-7-octadecenoate (69.02%) and methyl elaidate (16.87%). The essential oil contents of flowers and leaves of the Amanus sage plant were also determined by steam distillation method. When the essential oil components of the flower were examined, the main components were found to be tricosane (22.39%), caryophyllene (22.17%), alpha-cubebene (14.4305), beta-farnesene (7.7%) and linalool (4.69%). On the other hand the main components in the the leaves were caryophyllene oxide (27.20%), fenchone (18.63%), 1,8-cineole (6.12%), camphor (4.82%), and alpha-cubebene (4.11%). Bioactive properties of the components of Amanus sage obtained from both the ultrasonic extraction and steam distillation methods showed that this plant has important medicinal and aromatic properties.

Introduction

There is a growing interest for the demand of the utilization of medicinal and aromatic plants as new therapeutic agents and as a source for valuable biocides. Essential oils obtained from plants are used in many fields including food, spices, food preservation, folk medicine, pharmaceutical industry, and fragrance due to their antifungal, antibacterial, antiviral, and insecticidal properties (El Mokni *et al*. 2019).

Lamiaceae is one of the largest and most widespread families around the world including 236 genus and 6900-7200 species. The majority of plants in *Lamiaceae* family are well known to have medicinal properties. *Phlomis*, a perennial plant genus belonging to *Lamiaceae*, is known to have medicinal properties. Recently, The Turkish flora Phlomis genus were found to contain 34 species and 10 hybrids by Huber (1982). Twenty two of the 34 species are known to be endemic (Huber 1982, Çelik *et a*l. 2005, Aghakhani *et al*. 2018).

Phlomis species are divided into two main genera, *Flomoides* and *Phlomis*, and contain more than 100 species including *Flomoides* and *Phlomis* (Moench 1874, Albaladejo *et al*. 2015). *Phlomis* includes three genera, namely Dendrophlomis, Gymnophlomis, and Oxyphlomis and is mainly found in the Mediterranean region. Turkey is considered to be one of the primarily habitats of these main genus types (Huber 1982, Hedge 1986). Amanus Sage (*Pholomis longifolia* var. *longifolia*) Boiss. & Bl.) is an endemic plant species with a very narrow distribution around the Hatay-Belen (Turkey) region. It is located in the C6 corridor in the geographical distribution (Yüzbaşoğlu *et al*. 2009). Recent studies showed that main components of the essential oils extracted from different Phlomis species collected in Turkey and some countries contain hexa-hydrofarnesyl acetone, spathulenol, germacrene D, β-caryophyllin.

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Phlomis endemic species under the family Lamiaceae contribute as new alternative natural biocide sources with remarkable medicinal properties. The present study was undertaken to determine the chemical compositions of essential oils found in the leaves and flowers of Amanus sage plant and also to reveal the medicinal properties of the determined volatile components by using literature search found in some important databases including PubChem, Sigma Aldrich and Pharmacy Research Databases.

Material and Methods

The flowered aerial parts of *Pholomis longifolia* var. *longifolia boiss.* & Bl were collected in Turkey, C6 Hatay, Belen district, on rocky slopes, 840 m above the sea level, at the middle of April 2018. Species identification was made by Turgut Yılmaz, Director of Flora of Turkey Site, and the plant is included in the book of Ocak *et al.* (2014).The plant material was shade-dried at room temperature to get constant weight and weighed approximately 50 g as flowers and 40 g as leaves. Methanol (Isolab, catalog # 947.043.2500) and ethyl acetate (Tekkim, catalog # TK.050140.02500) were purchased and used as received.

Extraction of Amanus Sage plant was performed according to the ultrasonic-assisted solvent extraction method (input power 180 W, 35 kHz frequency at 25 ° C) using an ultrasonic bath as described in the literature (Heravi *et al.* 2009, Göktürk and Asil 2018). Extraction of the Amanus Sage sample was carried out as follows: sage flower and leaf were mixed and grounded. one g of the obtained mixture was then put in a flask. Ultrasonic extraction method was used to extract essential oils by using ethylacetate : methanol mixture, and GC-MS analysis was used to determine chemical composition.

The flask was charged with a mixture of (42–18 ml) methanol: ethylacetate (70:30) mixture as the extraction solvent and sonicated. Sonication was performed for 15 min. After sonication, orange colored organic extract was put into a centrifuge tube and centrifuged in 5000 rpm for 3 min. Obtained extract was collected. The 10 ml of the solvent mixture was then added to the residual, and sonicated for 10 more min. New formed organic extract was centrifuged for 3 min at 5000 rpm. After decantation, the organic extracts were combined together and concentrated up to 10 ml. Then, the final extract was stored at 4^{o}C in the fridge and the absence of light before GC–MS analysis. 1 µl of the extract was used for GC–MS analysis (Heravi *et al.* 2009, Göktürk and Asil 2018).

The essential oils of the *Pholomis longifolia var. longifolia boiss*. & Bl were obtained from the plant's fresh flowers (50 g) and leaves (50 g) by the steam distillation method. Steam distillation was carried out using Clevenger system for 3 hrs. The obtained essential oils were stored in dark glass bottles at $+4^{o}$C for further analysis (Turkmen 2021).

GC–MS analyses were performed using a Hewlett-Packard 6890 gas chromatograph equipped with a HP-5MS fused silica column (5% phenyl methyl polysiloxane 60 m 0.25 mm i.d., film thickness 0.25 µm), interfaced with a Hewlett-Packard mass selective detector 6890.

GC-MS analysis was performed according to the procedure described in the literature. The oven was first heated at 60°C for 2 min. The oven temperature was increased at 180°C by 4^{o}C/min. The temperature was then increased at 210°C by 2^{o}C/min and increased at 230°C by 2^{o}C/min and waited for 1 min. Finally, the oven temperature was increased at 280 by 2 ^{o}C/min. Helium (99.9999%) was used as the carrier gas and at a flow rate of 1 ml / min. The injector temperature was kept at 200°C. The separation rate was 1: 5 (Asil 2021, Asil and Göktürk 2021).

Results and Discussion

The potential drug, pharmacological and bioactivity properties of the obtained volatile components of Amanus Sage plant were investigated by literature search using PubChem, Sigma Aldrich and Pharmacy Research Databases (Anonymous 2021a,b).

A total of 20 volatile components were detected from the extracts of the Amanus sage plant by GC-MS analysis. The match factors of the obtained components found in GC-MS library and their total peak areas are summarized in the Table 1. The principal components of the obtained extract were found in the order of methyl-7-octadecenoate, methyl elaidate and methyl stearate (Table 1). Obtained 8 volatile components were found to have potential characteristics of Drug, Pharmacology, and Food.

Table 1. Chemical components ultrasonic extraction of Amanus Sage.

No	Chemical name	Formula	RT (min)	(%)	Activities
1	(E)-beta-ocimene	$C_{10}H_{16}$	6.16	0.25	Pharmacology, Food
2	1-Methyl-2-pyrrolidinone	C_5H_9NO	9.69	0.89	Food, Drug, Pharmacology
3	2-Tridecanol	$C_{13}H_{28}O$	14.26	0.52	Food
4	1-Tetradecene	$C_{14}H_{28}$	20.83	1.01	Pharmacology
5	(E)-5-Octadecene	$C_{18}H_{36}$	26.85	0.81	NF
6	2-Hexadecanol	$C_{16}H_{34}O$	32.32	0.38	NF
7	Methyl cyclohexanepropionate	$C_{10}H_{18}O_2$	36.80	0.11	NF
8	Methyl linoleate	$C_{19}H_{34}O_2$	46.13	1.98	Food, Pharmacology
9	Methyl-7-octadecenoate	$C_{19}H_{36}O_2$	46.78	69.02	NF
10	Methyl elaidate	$C_{19}H_{36}O_2$	47.08	16.87	NF
11	Methyl stearate	$C_{19}H_{38}O_2$	48.74	4.77	Food, Pharmacology
12	Methyl oleate	$C_{19}H_{36}O_2$	59.45	0.53	Food
13	Methyl-10-octadecenoate	$C_{19}H_{36}O_2$	59.50	0.70	NF
14	Methyl arachidate	$C_{21}H_{42}O_2$	61.00	1.12	NF
15	Methyl-9-hydroxy-nonanoate	$C_{10}H_{20}O_3$	61.14	0.21	Food

RT: retention time in minute. % Relative area per cent (peak area relative to the total peak,

Essential oil fraction of the flowers of Amanus Sage plant grown in the Hatay region was determined to be 0.015% in the dried flower sample. The essential oil components and their fractions in the flowers are presented in Table 2. The main essential oil components obtained from the flowers were tricosane (22.39%), caryophyllene (22.17%), alpha.-cubebene (14.43%), beta-farnesene (7.7%) and linalool (4.69 %). Eight of these volatile components were used in a variety of purposes including Drug, Pharmacological, and Food applications (Table 2).

The essential oil fractions of the leaves of Amanus sage were determined to be 0.002 % in the dried leaves sample. The essential oil components obtained from the leaves of the Amanus Sage plant are summarized in Table 3. The main volatile components obtained from the leaves of the Amanus sage were caryophyllene oxide (27.20%), fenchone (18.63%), 1,8-cineole (6.12%), camphor (4.82%), and alpha-cubebene (4.11%). Eight of these volatile components were used in a variety of purpose including Drug, Pharmacological, and Food applications (Table 3).

Table 2. Chemical components found in essential oil of the flowers of Amanus Sage plant.

No	Chemical name	Formula	RT (min)	(%)	Activities
1	alpha.-Cubebene,	$C_{15}H_{24}$	39.92	14.43	NF
2	Copaene	$C_{15}H_{24}$	41.91	2.44	NF
3	beta.-Cubebene	$C_{15}H_{24}$	46.29	4.19	NF
4	Linalool	$C_{15}H_{24}$	48.92	4.69	Food, Drug, Pharmacology
5	Caryophyllene	$C_{15}H_{24}$	51.10	22.17	Food, Drug, Pharmacology
6	beta-Farnesene	$C_{15}H_{24}$	51.45	7.77	Pharmacology
7	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	$C_{15}H_{24}$	53.29	1.25	NF
8	Humulene	$C_{15}H_{24}$	55.69	3.05	Pharmacology
9	2-(3,7-Dimethyl-octa-2,6-dienyl)-4-methoxy-phenol	$C_{17}H_{24}O_2$	70.48	1.20	NF
10	Tricosane	$C_{23}H_{48}$	72.79	22.39	Pharmacology
11	Fitone	$C_{18}H_{36}O$	74.02	4.21	Food
12	1-Chloro-3-(phenylsulfanyl)-2-butanone	$C_{10}H_{11}ClOS$	74.15	0.80	NF
13	Androstane, (5α)-	$C_{19}H_{32}$	74.49	5.32	Pharmacology
14	(E)-1-(2,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-7-Oxabicyclo[4.1.0] heptane	$C_{15}H_{24}O$	74.64	2.89	NF
15	Hexacosane	$C_{26}H_{54}$	76.30	3.29	Pharmacology

RT: retention time in minute. % Relative area percent (peak area relative to the total peak,

Table 3. Chemical components found in essential oil of the leaves of Amanus Sage plant.

No	Chemical name	Formula	RT (min)	(%)	Activities
1	1,8-Cineole	$C_{10}H_{18}O$	32.56	6.12	Food, Drug, Pharmacology
2	alpha-Cubebene,	$C_{15}H_{24}$	39.92	4.11	NF
3	Copaene	$C_{15}H_{24}$	41.91	1.84	NF
4	1-Octen-3-ol	$C_8H_{16}O$	43.07	2.75	Food, Pharmacology
5	.beta.-Cubebene	$C_{15}H_{24}$	46.30	2.51	NF
6	Fenchone	$C_{10}H_{16}O$	47.64	18.63	Food, Pharmacology
7	Linalool	$C_{15}H_{24}$	48.90	1.59	Food, Pharmacology
8	Caryophyllene	C_9H_{14}	51.10	27.20	Food, Drug, Pharmacology
9	1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1α,7α,8aβ)]- Azulene	C15H24	53.28	1.52	NF
10	Camphor	C10H16O	56.56	4.82	Food, Drug, Pharmacology
11	5-Nonen-2-one	C9H16O	72.38	2.44	NF
12	(5α)- Androstane	$C_{19}H_{32}$	72.88	1.04	Pharmacology
13	6-Methyl-2-tridecanone	$C_{14}H_{28}O_2$	73.98	2.90	NF
14	4,4-dimethyl-8-methylene-1-Oxaspiro[2.5]octane	$C_{10}H_{16}O$	75.82	1.56	NF
15	Bis(4-methylphenylthio)-methane	$C1_5H_{16}S_2$	89.48	2.13	NF
16	5 beta,14 beta-Androstane	$C_{19}H_{32}$	95.59	2.54	NF

RT: retention time in minute. % Relative area percent (peak area relative to the total peak,

There is a growing interest and research for the determination of volatile and bioactive components of medicinal and aromatic plants. There is still more efforts needed to discover potential utilizations of these kinds of plants. The present study revealed potential volatile and bioactive components of the Amanus Sage plant. Further studies will unveil the possible utilization of these components as drug, pharmacological and bioactivity applications.

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