Advances in Biology & Earth Sciences
Vol.9, No.2, 2024, pp.278-285
https://doi.org/10.62476/abes9278

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF WORMWOOD (*ARTEMISIA ABSINTHIUM L.*), GROWING IN THE WESTERN REGION OF AZERBAIJAN

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Abstract. Wormwood (*Artemisia absinthium* L.) is a widespread plant species in the Western region of Azerbaijan. The dependence of the composition, quantity and growth of various biologically active substances contained in the above-ground parts of wormwood on the vegetation period and climatic conditions was studied. The results of the study of the essential oil obtained by the steam distillation method from wet and dry samples of the aerial parts of bitter wormwood are presented. The yield of essential oil is determined in % in terms of the weight of air-dried raw materials. The study of the yield of essential oil was different months of the year showed that its amount varies depending on the vegetation period (yield of 0.56-0.66% by mass of fresh raw materials; yield of 0.58-0.77% by mass of dry raw materials). The composition of volatile distillates was studied by the chromatography method. In all samples, the amount of camphor component, which belongs to oxygenated terpenoid hydrocarbons, prevailed with a percentage above 35%. 25.44-34.24% phase budding in June for fresh and dry plant material; 25.14-35.21% phase mass flowering in August; it was determined between 25.67-35.20% phase the seed ripening in September.

Keywords: *Wormwood, essential oil, biologically active substance, vegetation, camphor.*

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Received: *2 April 2024;* ***Accepted:*** *5 June 2024;* ***Published:*** *2 August 2024.*

1. Introduction

In modern times, the scientific-research works carried out in the direction of the selection of useful plants, the detection of species containing biologically active substances and the discovery of new areas of application in pharmacology and cosmetology are always relevant (Abad *et al*., 2012).

Artemisia L. – Wormwood genus of the family Asteraceae Barcht.et J. Presil occupies a special place among the naturally distributed essential oil medicinal, food, fodder and agricultural plants in the flora of Azerbaijan. In the flora of Azerbaijan, Astaraceae Barcht.et J. Presil family is represented by 5550 species united in 125 genera. The largest genus of the family included in our study is *Artemisia* L. – Wormwood. The main beneficial properties of these plants are related to their chemical compounds. It is widespread in Azerbaijan in the Greater and Lesser Caucasus in the Kura-Araz plain, the

How to cite (APA):
Suleymanova, A.B., Aliyeva, K.T., Nasirova, A.E., Abbasova, S.H. & Mammadova, R.A. (2024). Chemical composition of the essential oil of wormwood (*Artemisia absinthium L*.), growing in the Western region of Azerbaijan. *Advances in Biology & Earth Sciences*, *9*(2), 278-285 https://doi.org/10.62476/abes9278

Lankaran plain, in Absheron, in the outer sand dunes of the Caspian Sea, in the middle mountain belts and in gravel sand dunes.

At present, plants belonging to the genus Artemisia L. have attracted wide interest from local and foreign researchers, not only because of the diversity of their species, but also because they contain various valuable biologically active substances and show activity in pharmacological fields (Bora & Sharma, 2011; Creed *et al*., 2015).

In the research work, the chemical composition of bitter wormwood (*Artemisia absinthium* L.), which grows wild in the Gazakh district of the Western region, was studied. Wormwood can be found on dry stony slopes of all regions of the Western region of Azerbaijan. Currently, bitter wormwood is actively spreading in the steppe and plain zones of Gazakh region and expanding its range. Wormwood is a tall plant with long leaves, the number of flowers in the basket is small. The particles of the leaves of the middle and upper branches are narrowly linear and the trunk is reddish-brown. It blooms in August, seeds in September-October (Kazemi *et al*., 2010).

The authors (Tabancha *et al*., 2011) during the morphological and cytological study of wormwood (*Artemisia* L.) common in Turkey found that bitter wormwood is morphologically different from other wormwood species. These morphological differences include length of stems, flowering, size of leaves, stamens, ovaries, direction of synflorescent branches, direction of heads on branches, color of corolla and types of fringes, etc. belongs to. At the same time, they differ by the number of chromosomes (Khodakov & Kotikov, 2008).

Wormwood is a whitish or gray plant due to the presence of dense, cobweb hairs that persist until the end of the growing season. It is a perennial herb with a height of up to 2 m. The roots are vertical, woody, thick and short. Shoots and ribbed trunks reach a height of 15-40 cm, consisting of short, slightly elongated branches with a curved shape at the top (Liu *et al*., 2013). Flowering is feathery, the thick gray-hairy leaves are convex, the edges are membranous, the outer ones are oval and the inner ones are larger, they consist of 6-8 lancet-shaped flowers. The seeds are ovoid, flat, gray and finely pointed, up to 1 mm long (Liu *et al*., 2010).

In the research work of the authors (Zovari *et al*., 2010), it was noted that the essential oil of wormwood growing in Tunisia contains monoterpenes: sabinene, myrcene, 1,8-cineole, α- and ß-thujones, as well as borneol, where thujones predominate. At the same time, there are species of this plant with different chemical compositions, which contain different amounts of nerol, neral, geranol and geranial in their essential oils (Gürkanli *et al*., 2018). Sesquiterpene-like compounds (tauremycin, mycbuloctane and taurine) chemically related to lactones have been identified in this type of wormwood. Despite the wide study of the taxonomy of the genus *Artemisia* L. in Azerbaijan, there is no information on the analysis of the chemical composition of its species, bitter wormwood. The purpose of this work is to study the chemical composition of the essential oil obtained from the above-ground parts of bitter wormwood growing wild in the natural biocenoses of the Western region during the growing season and to determine the quantity of its main components (Naseri *et al*., 2011).

2. Material and methods

Plant material

The aerial part of the bitter wormwood plant growing wild in the biocenoses of Gazakh region (43°05′ 35" N.E. 46°21′ 59" N.N.) was collected in the second week of every month during the months of June-August-September 2023. Plant material was analyzed in fresh and dried form. Dry raw materials were purchased in accordance with the rules of collection and drying of medicinal plants (Orav *et al*., 2006). In order to prevent the destruction of biologically active substances and to remove excess moisture, the raw materials are dried and stored in a dark place immediately after collection by a widespread method - air drying based on the free entry of air into the plant material (Shah *et al*., 2011).

Extraction of essential oil by steam distillation method

The above-ground part of bitter wormwood (500 g) was ground to 2-3 mm with a laboratory mill (SM-450L, MRCLab, Israel). From fresh and air-dried ground samples, essential oil was obtained by steam distillation method with Clevenger type apparatus according to European pharmacopoeia 35 for 4-5 hours (Taherkhani, 2015). The obtained oil was dried with anhydrous sodium sulfate and separated from the dryer by decantation. It was stored at 4ºC until use. The duration of the steam distillation process was determined experimentally based on the study of the dynamics of the yield of essential oil over time. The yield of essential oil was determined as a percentage (%) based on the weight of wet and dry raw materials.

Chemicals

All chemicals and solvents used in this study were purchased from Sigma Aldrich (St. Louis, MO).

Gas chromatography

The qualitative composition of the essential oil was determined by gas chromatography on an AutoSystem XL (Perkin Elmer, Canada) flame ionization detector chromatograph. In a 100 m long thin quartz capillary column (diameter 250 µm x 0.5 µm) it evaporates at a temperature of 250°C. Under the influence of the carrier gas (helium) constantly flowing through this tube, the essential oil in the form of vapor moves through the tubes. At the same time, the temperature of the column is 3-4°C/min and rises rapidly from 50°C to 200°C. In the samples, the analysis of individual components and the percentage composition were calculated according to the areas of the gas chromatographic peaks without using correction factors (Vieira *et al*., 2017).

Determination of physico-chemical properties of essential oil.

The main physico-chemical properties of essential oil: color, transparency, smell and taste, density (D^{20}_{20}), refractive index (N^{20}_{D}), kinematic viscosity, acid number (p. e.), ether number (p. e.), after acetylation is ether number (a.s.e.e.). Color was determined by physical observation in daylight and under UV light using an ultraviolet camera. Odor was determined by organoleptic evaluation after Evans (2002). Fat percentage was calculated according to AOAC, 2000. The refractive index was determined using a refractometer (model RL1 8056, Russia) and the kinematic viscosity was determined using a viscometer (model CT 72/P, Germany).

3. Results and discussion

Essential oil characterization

The moisture content of the above-ground part of bitter wormwood was calculated using the following formula according to the weight loss after the drying process (Valles *et al*., 2017):

$$Wet(\%) = \frac{Ci - Cn}{Cn} * 100\%$$

In the formula C_i – mass before the drying process or initial mass; C_n – mass after the drying process.

It was calculated based on the given formula that the moisture content of the sample is 2.3%.

The amount of oil obtained from each sample (fresh and dry) by the steam distillation method (%) was calculated by the following formula (Vieira *et al*., 2017):

$$\text{Oil } (\%) = ((M_2 - M_1) / m) \times 100$$

In the formula M_1 – mass of receiver brought to constant weight (g); M_2 – mass of receiver with oil (g); m – the mass of the received sample (g).

The study of the dependence of the yield of essential oil on the vegetation period showed that the highest yield was observed during the flowering period of the plant (Lima *et al*., 2011). The essential oil obtained with a high yield by the steam distillation method is a volatile liquid with a light-yellow color, a pleasant aroma, but a specific herbal smell. Table 1 presents the extract of essential oil obtained by mass of fresh and dried samples of bitter wormwood.

Table 1. The yield of essential oil from bitter wormwood depending on the vegetation period

Vegetation period	Yield of essential oil by of fresh sample, %	Yield of essential oil by mass of dry sample, %
June (Budding phase)	0.56	0.58
August (Mass flowering phase)	0.66	0.70
September (Seed ripening phase)	0.61	0.63

As can be seen from the table, the mass of 500 g of bitter wormwood in the flowering phase is determined by 0.66% of the essential oil obtained from the wet samples of the above-ground part and 0.70% of the essential oil obtained from the fresh samples. From this, it can be concluded that the amount of oil obtained during the flowering phase is higher than in other vegetation periods. In August, i.e. during the period of mass flowering, the reason for the increase in the amount of essential oil is due to the end of the complete formation of flowers.

Extraction of oil from bitter wormwood at different vegetative stages has been the subject of many researchers worldwide. Thus, according to Khodakov and Kotikov (2008) the essential oil from bitter wormwood, which grows wild in the Crimea, reaches its maximum in the second and subsequent years of the plants' life or at the beginning of the flowering period - mass flowering and the amount of oil obtained is 0.46 It was -48%.

Therefore, in conclusion, it seems that the bitter wormwood plant grown in the Western region can be a productive source of oil.

As a result of the study, the component composition of the essential oil obtained from bitter wormwood was determined by gas chromatography analysis (Table 2). As can be seen from the table, the amount of camphor component related to oxygen-containing terpenoid hydrocarbons in the essential oil obtained from dry and wet samples during the phase of mass flowering was determined to be 25.14% and 35.21%. The reason for the increase in the amount of the camphor component in the chemical composition of the essential oil is the persistence of the plant in hot climates. At this time, the plant under stress in the hot climate begins to secrete camphor in high quantities. At the same time, eucalyptol (31.57%-32.9%), the component containing the highest amount of monoterpene hydrocarbons, prevailed in the oil obtained in August.

Table 2. Chemical composition of essential oil obtained from bitter wormwood by gas chromatography method (percentage values)

Components	**June (Budding phase)**	**August (Mass flowering phase)**	**September (Seed ripening phase)**
Fresh samples			
α-Pinene	2.28	2.38	2.33
Comphor	25.44	25.14	25.67
Camphene	1.48	1.54	1.50
trans-Sabinene hydrate	1.45	1.50	1.39
cis-Sabinene hydrate	2.25	1.98	2.11
Eucalyptol	33.12	32.9	33.67
Thujone	7.78	7.98	7.88
Borneol	8.98	8.67	8.12
trans-Caryophyllene	13.09	13.68	13.43
Terpen-4-ol	4.13	4.23	3.9
SUM	**100**	**100**	**100**
Dry samples			
α-Pinene	0.33	0.40	0.35
Comphor	34.24	35.21	35.20
Camphene	1.57	1.78	1.67
trans-Sabinene hydrate	0.54	0.65	0.50
cis-Sabinene hydrate	1.98	1.18	1.11
Eucalyptol	31.12	31.57	30.12
Thujone	6.89	6.45	7.14
Borneol	7.78	6.88	8.11
trans-Caryophyllene	12.34	12.45	12.56
Terpen-4-ol	3.21	3.43	3.22
SUM	**100**	**100**	**100**

Physico-chemical properties of essential oil

Determination of various physicochemical properties investigates the practical importance of substances and justifies the utility of various vegetable oils in everyday life and medicine. In general, the economic importance of oils depends more on these physico-chemical indicators, which provide initial information to determine their suitability for consumption (Yousefzadeh, 2012). The essential oil obtained with a high yield by the steam distillation method is a volatile liquid with a light-yellow color, a pleasant aroma, but a specific herbal smell (You *et al.*, 2015). The results of physico-chemical analysis of oil obtained from wet and dry samples of bitter wormwood at different vegetation stages are given in Table 3. Determination of physico-chemical constants of essential oils was in accordance with the State Standard (Zanousi, 2016). As can be seen from Table 3, the essential oil number is higher than the acid number. A higher number of ethers indicates that they contain complex ethers and free alcohols formed from fatty acids and aliphatic alcohols.

Table 3. Physico-chemical parameters of wormwood oil in different vegetation period

No	Color	Trans-parency	D^{20}_{20}, g/sm^3	N^{20}_D,	Kinematic visco. 20°C, mm^2/s	Iodine number	Ether number	Acid number	Acetyl. then E.N.
N1	Yellow	Clear	0.9478	1.3789	1.7845	0.25	26.17	3.45	156.18
N2	Yellow	Clear	0.9456	1.3214	1.7569	0.09	48.78	5.46	199.88
N3	Yellow	Clear	0.8642	1.3897	1.8954	0.23	52.45	7.98	176.38
M1	Yellow	Clear	0.8953	1.3784	1.7896	0.22	25.87	6.28	256.34
M2	Yellow	Clear	0.9654	1.6278	1.7895	0.12	89.35	10.34	195.06
M3	Yellow	Clear	0.9785	1.4446	1.8964	0.09	70.64	8.67	230.75

Note: N – fresh samples, **N1** – Budding period, **N2** – Mass flowering period, **N3** – Seed ripening;
M – dry samples, **M1** – Budding period oil, **M2** – Mass flowering period oil,
M3 – Seed ripening period oil

4. Conclusion

In the conducted research, the qualitative and quantitative composition of the essential oil obtained from wormwood growing wildly in the Gazakh region of the Western region was chemically determined. The specific composition of the essential oil obtained from the studied plant depends on its species, as well as the soil and climatic conditions in which the plant grows.

In conclusion, we can note that due to the yield of essential oil (0.66-0.70%) obtained from the above-ground part of bitter wormwood during mass flowering, it can be used as a raw material source. Based on the therapeutic properties of the biologically active substances contained in wormwood, the oil obtained can be used in medicine and cosmetology for the preparation of ointments and lotions with various compositions. At the same time, the amount of camphor component, which belongs to oxygenated terpenoid hydrocarbons, which is a biologically important compound, in the essential oil obtained in August is 35.21%. Wormwood essential oil can be used as a potential natural source for cosmetology and pharmaceutical industry.

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