

# Essential oil composition of *Artemisia abrotanum* L. during different vegetation stages in Lithuania

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*Artemisia abrotanum* L. was introduced in the Middle of Lithuania Collection of Spice–Melliferous Plants of the Scientific Sector of Medicinal and Aromatic Plants of the Botanical Garden *ex situ* at Vytautas Magnus University since 1980. The object of investigation was *Artemisiae abrotani herba* of *Artemisia abrotanum* L. All samples were collected at different vegetation stages: growth and leaf production, flower bud development, the beginning of flowering, massive flowering and the end of flowering. Essential oils were obtained by hydrodistillation. The chemical composition of the essential oils of *Artemisiae abrotani herba* was studied by GC/MS in the Department of Analytical and Toxicological Chemistry at the Lithuanian University of Health Science in 2018–2019. Fifty-six compounds of the oil were identified, of which (+)-piperitone was the major component (20.38–38.48%). The data in this study showed a remarkable quantitative variation of constituents in the essential oils. The highest content and diversity of compounds was determined during the flower bud development stage. Sixty identified compounds in this stage reached 76.6% of the identified oil content. The highest concentration of this compound (38.48%) was detected at the end of the flowering vegetation stage and the lowest amount (20.38%) was found during the growth and leaf production. Among the other major compounds were (+)-piperitone, 1,4-cineole, lavandulyl butyrate, aromandendrene and isogermacrene D.

**Keywords:** *Artemisiae abrotani herba*, essential oils, chemotype, (+)-piperitone, vegetation stages, Lithuania

## INTRODUCTION

Essential oils are natural products, derived from medicinal, spice and aromatic plants (MAPs), traditionally used all over the world for disinfection, as anti-inflammatory, relaxing and stimulating substances, and with potential and modern exploitation in medicine. The chemical composition of essential oils varies widely depending upon the plant species, geographical location, botanical

origin, genetics and extraction techniques [1, 13, 16, 20]. During the past years, a number of studies have been carried out concerning the application of essential oils and active compounds from *Artemisia* L. genus. A lot of species of *Artemisia* L. are mentioned in folk and modern medicine, in the cosmetic and pharmaceutical industry, and they received special attention for their content of artemisinin which are active molecules against malaria [1, 9].

The object of investigation was *Artemisia abrotanum* L. (southernwood), a perennial medicinal,

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spice, aromatic semi-shrub of *Asteraceae* (Bercht. & J. Presl) family, collection number XX-0-KAUN-1980-AR0025, coordinates on the map 54.870453, 23.908354. *A. abrotanum* is widely used in traditional medicine for treating a variety of disorders, including upper airway diseases [4]. Moreover, it has been found to possess spasmolytic activity on the carbacholine-induced contraction of guinea pig trachea [3, 5, 7, 12]. Nowadays, this perennial plant is used mainly for culinary or cosmetic purposes [1].

*A. abrotanum* is an essential oil accumulating plant [1, 11]. Essential oil is accumulated in the overground raw material. It has been determined that *A. abrotanum* growing in different countries is dominated by monoterpenes such as camphor and 1,8-cineole which are representative components in the investigated oils, which were reported to exhibit antimicrobial and antimicrobial activity [8, 14, 19]. The oils from Cuba contained *trans*-sabinyl acetate (33.4%) and  $\alpha$ -terpineol (8.2%) [17]. German southernwood oil is rich in 1,8-cineole,  $\alpha$ -thujene and  $\alpha$ -pinene [21]. Eucalyptol was determined as the major constituent in *A. abrotanum* from Romania [17]. According to the published data, the oils of *A. abrotanum* are characterized by the heterocyclic sesquiterpenoids davanol, davanone and hydroxydavanone [9]. As studies show, extracts of *A. abrotanum* exhibit anticancer, expectorant, antiseptic, anti-inflammatory and antioxidant activities [1, 15, 17]. The analysis of *A. abrotanum* herb collected in Romania was presented in the Baiceanu et al. 2015 study. The results showed 19 polyphenols and flavonoids with high contents of sinapic acid, rutin, ferulic acid, luteolin and patuletin [6].

Considering different pharmacological effects of essential oil components it is very important to determine the chemotype and composition of the essential oil of *A. abrotanum* plants that are introduced in Lithuania. With many different chemotypes of *A. abrotanum* from diverse geographical locations, it is interesting to speculate that traditional medicinal uses may vary depending on the geographical location and the possibility of chemical compounds of different plant vegetation stages.

The aim of our study was to determine the qualitative and quantitative composition of the essential oils obtained from *Artemisia abrotani* herba

grown under Lithuania climatic conditions during different vegetation stages. At present, there is no data on the essential oil composition of *A. abrotanum* in Lithuania.

## MATERIALS AND METHODS

### Plant material

The research work presents details of the introduction of *Artemisia abrotanum* L. since 1980 in the Spice–Melliferous Plants Collection of the Scientific Sector of Medicinal and Aromatic Plants of the Botanical Garden at Vytautas Magnus University (VMU) and its phytochemical analysis results in the Department of Analytical and Toxicological Chemistry at the Lithuanian University of Health Science. *Artemisiae abrotani herba* of *A. abrotanum* was collected during different vegetation stages of the vegetation cycle in the Spice–Melliferous Plants Collection *ex situ* of the Botanical Garden at VMU in 2018. Five stages have been separated: growth and leaf production, flower bud development, the beginning of flowering, massive flowering and the end of flowering. The raw material (leaves, stems and flowers) of *A. abrotanum* was dried at 25°C temperature in a ventilated lodge avoiding direct solar radiation for 4 weeks. The dried material was stored in a dark room at ambient temperature and used for further analysis. Essential oils were obtained by hydrodistillation using a closed type Clevenger apparatus. In brief, 15 g of the dried material was mixed with 500 mL distilled water and submitted to extraction for 3 h until no more essential oils was obtained. The yield of essential oils was 0.01% (v/w). The white coloured oil with pleasant aroma was stored in a refrigerator at +4°C until further use.

### Gas chromatography, gas chromatography-mass spectrometry

The analysis of essential oils was performed using gas chromatography with a mass spectrometer detector (GC-MS-QP2010, Shimadzu, Tokyo, Japan) equipped with a Shimadzu autoinjector AOC-5000 (Shimadzu, Tokyo, Japan). Separation of compounds was performed on a RXI-5MS column (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m film thickness) (Restec, Bellefonte, USA). The operational conditions were as follows: temperature program from 50°C (5 min) to 200°C at 2°C/min and to

315°C (15 min) at 15°C/min. The GC-MS-QP2010 was equipped with a split/splitless injector (260°C). Split ratio: 1:60. Inlet pressure: 70.2 kPa. Carrier gas: helium (purity >99%) delivered at constant linear velocity of 40 cm/s. Interface temperature: 280°C. MS ionization mode: electron ionization. Detector voltage: 0.99 kV. Acquisition mass range: 29–500 u. Scan speed: 2500 amu/s. Acquisition mode: full scan, scan interval 0.20 s.

### Qualitative and quantitative analyses

The percentage composition of essential oils was computed from GC peak areas without correction factors. The qualitative analysis was based on a comparison of retention times, indexes and mass spectra with the corresponding data in the literature [2] and NIST/FFSNC computer mass spectra libraries.

## RESULTS AND DISCUSSION

### Determination of essential oil compounds in the southernwood samples

A preliminary study on the chemical composition of essential oils of *Artemisia abrotanum* L. was conducted in 2018. A total of 56 different compounds were found in *A. abrotanum* essential oils. The data showed that the amounts of compounds with the content of major constituents varied significantly from 0.04 to 38.48% (v/w). Of all the identified compounds, (+)-piperitone predominated during all vegetation stages. The highest concentration of this compound (38.48%) was detected at the end of the flowering vegetation stage and the lowest amount (20.38%) was during the growth and leaf production.

Amounts of all compounds are listed in the Table and expressed in arbitrary units of the peak area in a GC/MS chromatogram. The GC/MS peak area was selected for quantitative purposes because the interpretation by using a percentage composition would not be suitable due to a different number of compounds; anyway, the range of the percentage composition of all compounds at different vegetation stages is given.

The amount of identified compounds varied during different vegetation stages. Forty-four constituents of essential oil, representing 68.2% of identified compounds, were found in the essential oil of *A. abrotanum* at growth and leaf production

Table. Variation of the essential oil composition of *Artemisia abrotani herba* collected during different vegetation stages in Vytautas Magnus University Botanical Garden, 2018

Compounds	RI	Interval, %
*** (+)-3-Thujanol	927	0–0.21
(+)-Camphene	928	0.10–0.64
* $\alpha$ -Pinene	954	0.09–0.2
* $\beta$ -Myrcene	956	0.05–0.11
1-Octen-3-ol	965	0.16–0.18
*** $\beta$ -Ocimene	975	0–0.07
*** trans- $\beta$ -Ocimene	986	0–0.05
$\alpha$ -Terpinene	1000	0.21–0.31
$\alpha$ -Phellandrene	1011	0.05–0.25
* 1,4-Cineole	1013	4.12–13.14
*** 1,8-Cineole	1015	0–13.0
* $\beta$ -Phellandrene	1041	0.35–0.44
cis-Sabinene hydrate	1049	0.04–0.11
*** trans-Ocimenol	1069	0–0.08
$\alpha$ -Terpinolene	1070	0.07–0.09
* Linalool	1085	0.08–0.11
*** Nonanal	1103	0–0.33
* Camphor	1122	0.86–1.33
* cis-Chrysanthenol	1146	0.49–0.84
*** trans-Pinocamphone	1151	0–0.11
*** (-)-Lavandulol	1152	0–0.30
* 4-Thujanol	1157	0.52–1.0
*** cis-Piperitol	1190	0–0.17
* (+)-Piperitone	1241	20.38–38.48
* cis-Chrysanthenyl acetate	1244	0.15–0.21
trans-Sabinyl acetate	1275	0.29–0.50
* $\delta$ -Terpineol acetate	1297	0.10–0.14
$\alpha$ -Terpinyl acetate	1329	0.05–0.16
* $\alpha$ -Cubebene	1352	0.08–0.34
*** $\beta$ -Bourbonene	1360	0–0.52
(-)-Cembrene	1369	0.11–0.12
Z-Jasmone	1378	0.15–0.18
* cis-Arbusculone	1387	0.35–2.15
* Aromandendrene	1394	2.39–7.44
$\beta$ -Cubebene	1402	0.11–0.12
$\beta$ -Copaene	1403	0.09–0.13
$\alpha$ -Humulene	1426	0.32–0.51

Table. (Continued)

Compounds	RI	Interval, %
(E)- $\beta$ -Farnesene	1439	0.15–0.19
* Lavandulyl butyrate	1449	0.83–4.70
Isogermacrene D	1454	5.23–15.67
*** $\gamma$ -Amorphene	1458	0–17.85
*** Germacrene D-4-ol	1462	0–0.44
Bicyclogermacrene	1470	0.27–0.83
*** $\alpha$ -Selinene	1478	0–0.09
Lavandulyl isovalerate	1491	0.33–0.65
Artedouglasia oxide C	1510	0.20–0.23
Farnesyl butanoate	1542	0.32–0.38
* Isospathulenol	1550	0.26–0.80
*** epi-Longipinanol	1553	0–0.92
Caryophyllene oxide	1554	0.45–1.15
*** Davanone B	1564	0–0.67
*** E-Myrtenol	1566	0–0.90
*** $\alpha$ -epi-7epi-5-Eudesmol	1623	0–0.34
* Lavandulyl caproate	1635	0.28–1.21
* (6R,7R)-Bisabolone	1719	0.17–0.46
$\gamma$ -Eudesmol acetate	1742	0.80–2.18
<b>Total</b>		<b>80.4–95.7</b>
Monoterpene hydrocarbons		5.6–27.4
Oxygenated monoterpenes		14.3–47.8
Sesquiterpene hydrocarbons		13.6–44.5
Oxygenated sesquiterpenes		0.2–8.3

RI is the retention index; \* is the compound found in all vegetation stages; \*\*\* is the compound found only in one vegetation stage.

stages. The principal compounds were found to be (+)-piperitone (20.38%) and isogermacrene D (5.23%). Only in this vegetation stage we identified  $\alpha$ -terpinyl acetate (0.16%),  $\delta$ -terpineol acetate (0.10%) and davanone B (0.67%). Some of our identified compounds in *A. abrotanum* samples were also found by Pino et al. (2011); however, the quantitative distribution of the compounds was a bit different. In the study of Pino et al. (2011), the main compounds found in dried leaves were *trans*-sabinyl acetate (33.4%),  $\alpha$ -terpineol (8.2%), terpineol-4-ol (5.9%), *trans*-sabinol (5.1%) and sabinene (4.4%) [17].

The highest content and diversity of compounds have been obtained from *A. abrotanum* essential oil during the flower bud development stage. We de-

tected 60 constituents of essential oil, representing 76.66% of identified compounds. The principal compounds were found to be (+)-piperitone (25.36%),  $\gamma$ -amorphene (17.85%), 1,4-cineole (7.65%) and aromandendrene (7.44%).

Another two essential oils were obtained from *A. abrotanum* collected at the beginning of flowering and massive flowering vegetation stages. Isogermacrene D dominated in both oils (14.90%; 15.67%); the second main constituent was 1,4-cineole (8.15%; 13.14%) and the third component was aromandendrene (6.44%; 6.45%). In the beginning of flowering 57 constituents of essential oil were found, representing 61.41% of the identified compounds, or in massive flowering 59 constituents of essential oil, representing 61.02% of the identified compounds. In the end of flowering also 59 constituents of essential oil were found, representing 57.63% of the identified compounds. The principal compounds were found to be (+)-piperitone (38.48%),  $\gamma$ -amorphene (14.37%) and aromandendrene (5.82%).

As results show, (+)-piperitone has been found as the first principal component in all vegetation stages (20.38–38.48%). Camphor,  $\beta$ -myrcene, 1,4-cineole,  $\alpha$ -pinene,  $\beta$ -phellandrene, *cis*-chrysanthenol, lavandulyl butyrate, 4-thujanol, *cis*-chrysanthenyl acetate,  $\alpha$ -cubebene, lavandulyl caproate, aromandendrene, *cis*-arbusculone,  $\delta$ -terpineol acetate, (6R, 7R-bisabolone) and isospathulenol were extracted in all vegetation stages. E- $\beta$ -farnesene and  $\alpha$ -phellandrene were found in all stages, except for massive blossoming. (+)-piperitone, isogermacrene D, 1,4-cineole, lavandulyl butyrate and aromandendrene could be attributed as the main compounds of the essential oils of *Artemisia abrotanum* L.

## CONCLUSIONS

The chemical composition of essential oils of *Artemisia abrotanum* L. introduced *ex situ* in the Spice–Melliferous Plants Collection of the Scientific Sector of Medicinal and Aromatic Plants of the Botanical Garden at Vytautas Magnus University was investigated in 2018–2019. Fifty-six compounds of the oil were identified, of which (+)-piperitone was the major component (20.38–38.48%). The highest content and diversity of compounds were determined during

the flower bud development stage. Sixty identified compounds in this stage formed up 76.66% of the identified oil content. The highest concentration of this compound (38.48%) was detected at the end of the flowering vegetation stage and the lowest amount (20.38%) was found during the growth and leaf production. Among the other major compounds were (+)-piperitone, 1,4-cineole, lavandulyl butyrate, aromandendrene and isogermacrene D determinate for the first time in Lithuania.

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## DIEMEDŽIO (*Artemisia abrotanum* L.) ETERINIŲ ALIEJŲ CHEMINĖ SUDĖTIS SKIRTINGAIS VEGETACIJOS TARPSNIAIS LIETUVOJE

### Santrauka

Diemedis (*Artemisia abrotanum* L.) introdukuotas *ex situ* 1980 m. Vidurio Lietuvoje, Vytauto Didžiojo universiteto Botanikos sodo Vaistinių ir prieskoninių (aromatinių) augalų mokslo sektoriaus Prieskoninių ir medingųjų augalų kolekcijoje. Tyrimo objektas – vaisinė augalinė žaliava *Artemisiae abrotani herba*, paruošta tyrimams 2018 m. skirtingais vegetacijos tarpsniais: intensyvaus augimo, butonizacijos, žydėjimo pradžioje, masinio žydėjimo ir žydėjimo pabaigoje. Eteriniai aliejai išskirti hidrodistiliacijos metodu, jų sudėtis išanalizuota dujų chromatografijos / masių spektrometrijos metodais 2018–2019 m. Lietuvos sveikatos mokslų universiteto Farmacijos fakulteto Analizinės ir toksikologinės chemijos katedroje. Atlikus tyrimus identifikuoti penkiasdešimt šeši eterinio aliejaus junginiai. Ištyrus eterinių aliejų sudėtį nustatyta jų dinamika skirtingais vegetacijos tarpsniais: maksimalus kiekis butonizacijos – 60 junginių, iš jų identifikuota 76,66 %, minimalus intensyvaus augimo metu – 44 junginiai, iš jų identifikuota 68,2 %. (+)-piperitonas kaupiasi visais vegetacijos tarpsniais: maksimalus kiekis – žydėjimo pabaigoje (38,48 %), minimalus – intensyvaus augimo metu (20,38 %). Vyraujantys junginiai: (+)-piperitonas, 1,4-cineolis, lavandulilo butiratas, aromandendrenas, isogermakrenas D Lietuvoje identifikuoti pirmą kartą.