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Slovak Chamomile Varieties and their Comparison of Natural Components

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Abstract

Chamomile, *Matricaria recutita* L., is one of the most important medicinal plants cultivated in the Central Europe. Primarily the dry flower anthodia, *Chamomillae Flos*, in its entirety has anti-inflammatory, antiseptic, healing, simulative, carminative, spasmolytic and sedative activity. Slovakia belongs to these European countries in which particular attention has been devoted to the research and development of chamomile in all its aspects including the breeding of this special crop. Prof. Dr. Robert Honcariv was the pioneer of medicinal plant breeding in Slovakia, and made the first selections in chamomile at the P. J. Safarik University in Kosice. The first variety '*BONA*' was registered in the National Book Variety in 1984. The first tetraploid variety '*LUTEA*' was accepted by the Slovak Central Control and Testing Institute in Agriculture in 1995. Both these varieties were from the old century. Their qualitative and quantitative parameters are very commonly inconvenient. On the present, the chamomile variety '*LIANKA*' was bred at the University of Presov, Slovakia, between the years 2008 – 2013. Currently, this variety obtains the Certificate by the Community Plant Variety Office (CPVO) in Angers, France in 2018. The GC/MS results confirm earlier reports that major volatile constituents obtained from the flower inflorescences are /-/-α-bisabolol (67.35 ± 2.82 %) and chamazulene (10.05 ± 1.04 %) and the low contents of /-/-α bisabolol oxides A and B (2.95 ± 0.32 %).

Key Words: Composition, Essential Oil, Chamomile, Quality, Sesquiterpenes, Slovakia, Varieties

1. Introduction

Chamomile, *Matricaria recutita* L., is most favored and used medicinal plant in Slovakia. Our folk saying indicates that individual should always bow when facing a chamomile plant. This respect resulted from hundred years' experience with curing in folk medicine of the country.

In former Czechoslovakia, the first recommendation for chamomile cultivation was published in the book '*Cultivation of Medicinal, Aromatic and Spice Plants*' by Dr. Vladimir Mycak in 1953. The author referred to the results of his own chamomile cultivation experience. It is very interesting information about a time comparison in days between using hand (390 days) and horsy (14 days) work per 1 ha. The seeds (2 or 3 kg) or seedlings (180,000 or 200,000 pcs.) used for a large-scale cultivation in this time. The dry flower yield per ha was determined from 400 to 600 kg. On the other hand, whole production of Czechoslovak chamomile originated in collecting the wild growing plants approximately till the half of the fifties of XX century, when large-scale cultivation began to introduce. In the first stage, the seeds reproducing by self-seedling in country gardens in Bohemia and Moravia were firstly used for cultivation. In 1952, these seeds became the ground of the regional variety given as *Matricaria chamomilla* L., *forma culta provincialis Bohemica*, later known as the variety '*BOHEMIA*' (Jasicova and Felklova, 1979).

In Slovakia, the breeding activity became more intensified by involvement of the Department of Special Biology, P.J.Safarik University in Kosice, in the scientific project "*The Research of Drugs based on Medicinal Plants and Natural Substances*". From 1975 to 1995, under the leadership Prof. Dr. Robert Honcariv, the breeding works on varieties '*BONA*', '*GORAL*', '*NOVBONA*' and '*LUTEA*' were gradually accomplished. Also, the methods of conserving breeding of this plant material were elaborated (Salamon and Honcariv, 1994). In 2015 the new Slovak variety '*LIANKA*' was widely introduced into cultivation, which helped to supply large amounts of uniformed raw material of high quality for pharmaceutical purposes.

This study presents essential oil contents and their qualitative-quantitative composition of natural substances in two Slovakian varieties ('*LIANKA*' and '*LUTEA*'), which are cultivating under different field environments.

2. Material and Methods

A several years field experiment was initiated with a diploid variety '*LIANKA*' and tetraploid variety '*LUTEA*' at 2 localities in the Slovakia (Tab. 1): Trebisov (GPS: N 48° 38' 01"; E 21° 43' 02"; altitude: 109 m) – a warm climatic region of the Lowlands in East Slovakia with mildly acid black fertile soil having a high content of phosphorous, potassium, and magnesium; and Plavnica (GPS: N 49° 16' 28"; E 20° 46' 50"; altitude: 530 m) – with a mildly warm climatic montane region with neutral soil and good fertility No fertilizers were added to any of the soils at the experimental sites.

The essential oil was isolated from dry flower anthodia by repeated hydro-distillation (from 6 to 8 times depending on sample size) and the weight of oil was determined gravimetrically (Humphrey, 1992; Read, 1992). The modified distillation apparatus by Coocking & Middleton were used (9th ed. Ph. Eur., 2018).

The essential oil components were characterized using a GC-MSD system on a Varian 450-GC instrument together with a Varian 220-MS with Split-Splitless injection port, MSD detector. Two columns were used: RX-5MS (non-polar), 30 m × 0.25 mm with an inside diameter: 0.25 µm, carrier gas: helium (21 psi) with a flow rate of 1.50 ml.min-1 and a BPX-5MS column (polar), 50 m long with an inside diameter of 0.25 mm and a thickness stationary phase 0.25 µl. Temperature program: 50 °C - 0 min.; 3 ° $C.min^{-1}$ to 250 °C; 250 °C - 15 min.. The identification of the individual components of the essential oil was made using the retention times of 40 authentic component standards supplied by the companies: Extrasynthese, Merck, Fulka and Sigma-Aldrich, Kovat's indexes (used C_5-C_{22} alkanes) and NIST 14 integrated library (version 2014). The spectra of the individual constituents of essential oil were compared to mass spectra for using literature (Adams, 2007).

Several statistical methods and biometric parameters (Palaniswamy, 2006) were used to evaluate biological material (n = 6, 8) and chemical identification results: arithmetic mean, weighted arithmetic mean, standard deviation, mean error, and confidence interval after transforming the percentage by angular transformation.

3. Results and Discussion

The aim of Slovakian's breeding program and requirements of new chamomile variety were: high content of active substances (essential oil, /-/-α-bisabolol, chamazulene), sufficient yield of dry flower drug, morphological traits required for the harvest mechanization (increase of flower heads size and their number, synchronous flowering, long pedicle with leaves, high tubular flower frequency) and resistance to abiotic stress (drought and flood). For achievement of success in regard to the variety '*LIANKA*', it was chosen conventional breeding methods – selection and examination of posterity of selected plant by method of "the middle seedbed". In the case of individual selection there is necessary to take into account that it is the population of each other pollinating heterozygotes. Repeated long time lasting selection extreme individuals causes progressive decay of primary population to families (Rod, 1982).

The contents of chamomile oil isolated from samples of dry flowers grown in Trebisov (variety '*LIANKA*') and in Plavnica (variety '*LUTEA*') confirmed intervals of quantities ranging from 0.60 ± 0.20% to 0.64 ± 0.25% (Table 1).

The range of the confidence interval of the essential oil content shows that there are no significant differences between both varieties. Chamomile variety '*LUTEA*', as tetraploid plants, must present higher content essential oil, from 1.0 to 1.2 %, into the dry raw-material (Oravec Sr. and Oravec Jr., 2007). In regard to results from the field experiment this respect has not been validated.

Table 1. Geographic coordinates of experimental fields and content of essential oil in chamomile flowers

Locality / Variety	Geographic coordinate system Latitude / Longitude	Altitude above sea level	The yield of essential oil [%]
Trebisov variety '*LIANKA*'	N 48° 38' 01" E 21° 43' 02"	109 m	0.64 ± 0.20
Plavnica variety '*LUTEA*'	N 49° 16' 28" E 20° 46' 50"	530 m	0.60 ± 0.25

Full-scale of essential oil GC-MS profiles showed the identified 75 respectively 68 chemical parties in the essential oil isolated from the dry flowers both varieties (Table 2).

For the identification of individual chemical types, we can select only the main essential oil components, which are important not only for therapeutic properties but also for industrial processing in the pharmaceutical and cosmetic industries.

Dry chamomile flower heads of the variety '*LIANKA*', which were collected in 2018, contained in their essential oil the main components – /-/ - α-bisabolol: 67.35 ± 2.82 %, chamazulene: 10.05 ± 1.04 %, /-/ - α-bisabolol oxide A: 1.11 ± 0.28 %, /-/-α-bisabolol oxide B: 1.84 ± 0.32 %, cis-en-in-dicycloether: 8.88 ± 1.47 % and α-farnesene: 2.13 ± 0.44 % .

The essential oil of variety '*LUTEA*' involved /-/ - α-bisabolol: 42.53 ± 1.51 %, chamazulene: 18.34 ± 0.74 %, /-/ - α-bisabolol oxide A: 1.45 ± 0.31%, /-/ - α-bisabolol oxide B: 4.80 ± 0.81 %, cis-en-in-dicycloether: 20.55 ± 0.77 % and α-farnesene: 3.68 ± 0.53%. In regard to the results of essential oil composition, both varieties belong to the bisabolol chemotype (Lawrence and Reynolds, 1987); of course the variety '*LIANKA*' has higher content of /-/-α-bisabolol.

Table 2. Full-scale of GC-MS essential oil profiles of the variety '*LUTEA*' and '*LIANKA*'

			% area	
Natural components	***Rt* *[min.]**	**Kovat's Index**	**'*LUTEA*'**	**'*LIANKA*'**
trans-2-hexanal	10.28	850	0.05	0.12
tricyclene	10.35	885	0.07	0.14
α-pinene	13.44	933	0.18	0.10
camphene	14.42	941	0.05	0.17
sabinene	15.44	974	0.06	0.17
β-pinene	16.01	981	0.07	0.13
β-myrcene	17.49	986	0.05	0.08
p-cymene	18.36	1031	0.07	0.11
limonene	18.58	1034	0.08	0.13
cis-β-ocimene	19.04	1035	0.09	0.07
trans-β-ocimene	19.06	1038	0.06	0.15
cis, cis-allo-ocimene	19.19	1047	0.13	0.11
dihydro tageton	19.28	1061	-	0.06
γ-terpinene	19.36	1064	0.30	0.29
terpinolene	19.90	1093	0.10	0.12
nerol	19.98	1245	0.14	0.13
methyl geranate	20.03	1336	0.07	0.42
methyl acetate	20.46	1344	0.19	0.05
carvacryl acetate	20.49	1347	0.05	0.07
germacrene β	20.51	1349	0.05	0.07
neryl acetate	20.56	1371	-	0.04
α-copaene	20.69	1395	0.07	0.15
decanone	21.81	1406	0.07	0.03
decanoic acid	23.00	1413	0.51	0.35
longifolene	23.21	1440	0.05	0.11
β-caryophyllene	23.59	1443	0.25	0.04
α-guaiene	23.76	1452	0.06	0.08
trans-β-fernesene	23.91	1464	0.33	0.10
aromadendrene	24.68	1467	0.35	0.35
ε-cadinene	24.73	1475	0.17	0.13
α-caryophyllene	24.79	1481	0.21	0.17
allo-aromadendrene	24.96	1485	-	0.03
(ε,ε)-farnesyl acetate	25.01	1492	0.04	0.09
bicyklogermacrene	25.13	1499	0.12	0.08
germacrene D	25.21	1506	0.08	0.14
γ-muurolene	25.49	1509	0.05	0.27
α-farnesene	**25.83**	**1514**	**3.68**	**2.13**
α-muurolene	26.78	1520	0.04	0.29

α-bulnesene	27.11	1526	0.05	0.16
γ-cadinene	27.91	1540	0.05	0.04
calamenene	27.99	1545	0.01	0.06
α-acoradiene	28.02	1553	0.12	0.14
cadina-1,4-diene	28.24	1557	0.13	0.11
δ-cadinene	28.36	1564	0.62	0.13
α-amorphene	28.42	1566	0.08	0.07
α-calacorene	28.54	1568	0.09	0.11
trans-nerolidol	28.60	1571	0.09	0.40
epiglobulol	28.73	1585	0.06	0.12
junenol	28.81	1591	0.08	0.11
spatulenol	28.87	1602	0.48	0.09
β-eudesnol	29.07	1606	0.10	0.13
globulol	29.53	1610	0.15	0.11
tremetone	29.84	1612	0.12	0.09
α-bisabolon oxide A	30.26	1615	0.15	0.15
viridiflorene	30.29	1625	0.04	0.05
dillapiole	30.42	1642	0.03	0.08
cubebol	30.50	1655	0.07	0.06
β-bisabolol	30.56	1660	1.66	0.13
τ-muurolol	30.61	1668	0.15	0.11
α-bisabolol oxide B	**30.69**	**1678**	**4.80**	**1.84**
bulnesol	30.89	1683	0.01	0.03
valerianol	31.02	1687	-	0.01
α-farnesol	31.39	1691	0.08	0.07
α-bisabolone oxide A	32.27	1702	0.23	0.12
cadalene	33.13	1705	-	0.04
α-bisabolol	**33.52**	**1710**	**42.53**	**67.35**
chamazulene	**42.92**	**1772**	**18.34**	**10.05**
α-bisabolol oxide A	**44.20**	**1782**	**1.45**	**1.11**
guaiazulene	45.36	1806	0.14	0.06
(ζ, ε)-farnesyl acetate	46.09	1842	-	0.01
cis-en-in-dicykloether	**46.57**	**1928**	**20.55**	**8.88**
trans-en-in-dicykloether	47.99	1940	0.04	0.14
hexadekanoic acid	53.34	1965	0.08	0.22
eicosane	54.97	1999	-	0.03
total			**100.00**	**100.00**

*Rt *-retention times*

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2.13 ± 0.44 %. The essential oil of variety '*LUTEA*' involved /-/ - α-bisabolol: 42.53 ± 1.51 %, chamazulene: 18.34 ± 0.74 %, /-/ - α-bisabolol oxide A: 1.45 ± 0.31%, /-/ - α-bisabolol oxide B: 4.80 ± 0.81 %, cis-en-in-dicycloether: 20.55 ± 0.77 % and α-farnesene: 3.68 ± 0.53%.

In regard to the results of essential oil composition, both varieties belong to the bisabolol chemotype (Lawrence and Reynolds, 1987); of course the variety '*LIANKA*' has higher content of /-/-α-bisabolol. According to Table 3 the chamomile essential oils after a laboratory hydro-distillation were presented the highest levels of the precursor sesquiterpenes –/-/-α-bisabolol and chamazulene (70.27 respectively 84.50 %), ethers – cis- and trans-en-in-dicykloethers (9.02 respectively 20.59 %), sesquiterpene oxides (3.22 respectively 6.63) and level of monoterpenes were observed very low (≥ 4.0 %).

Table 3. Groups of natural components [%] identified in essential oil of '*LUTEA*' and '*LIANKA*' varieties

Group and compounds/Slovakian varieties	'*LUTEA*'	'*LIANKA*'
Monoterpenes (chiefly: α-pinene, β-pinene, β-myrcene, *p*-cymene, limonene, ocimenes, methyl acetate, decanoic acid)	2.37	3.13
Monoterpenes alcohols (chiefly: nerol)	0.14	0.13
Ether (chiefly: cis-en-in-dicycloether, trans-en-in-dicycloether)	20.59	9.02
Sesquiterpenes (chiefly: α-bisabolol, chamazulene)	70.27	84.50
Sesquiterpene oxides	6.63	3.22

Based on the study of pharmacodynamics properties, /-/-α-bisabolol and chamazulene are considered to be the most valuable constituents of this plant species (Isaac, 1979). Formulation several liquid and ointment preparations were standardized to contain essential oil, high contents of both natural substances (Mann and Staba, 1986). These formulations were used on support of the skin's ability to repair it, strengthen the tissues of the mouth's natural defense, sooths the throat and airways, and have a calming effect on the skin and digestive system (Shilcher, 2004).

4. Conclusion

Breeding efforts were successful in developing the chamomile diploid variety '*LIANKA*' of better essential oil composition than tetraploid variety '*LUTEA*'. The total oil content and /-/-α-bisabolol content of oil were increased. The variety has plant populations, which are characterized by good yield, resistance to stress, bigger flower heads, and sufficient amount of essential oil of appropriate chemical composition. Cultivated raw material has totally displaced wild collection of chamomile flower heads. The chamomile variety '*LIANKA*' and its large-scale cultivation, extraction and distillation is very forward raw-material to the today's pharmaceutical industry.

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Conflict of Interest

I may declare that I have not any conflicts of interest.

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