

Research Article



Study of Economically Valuable Features of *Nepeta grandiflora* M. Bieb. Genotypes in the South Steppe of Ukraine

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	Article Details:		
BY NC	Received:	2024-10-08	
	Accepted:	2024-11-15	
	Available online:	2024-11-30	

DOI: https://doi.org/10.15414/ainhlq.2024.0022

Nepeta grandiflora M. Bieb. known as aromatic, medicinal, and ornamental plants, whose extracts contain biologically active compounds. The essential oil of this species is a rich source of valuable components. This study aimed to evaluate plants' biological features and the biochemical content of the essential oil of N. grandiflora genotypes grown in the South of Ukraine. Genotypes G1-21, G2-20, G3-22, G4-20, and G5-21 were investigated for morphometric and some biological features in the flowering stage. In the genotype, plants were measured the length and width of inflorescences (in cm), the number of whorls in the inflorescences, the number of flowers in the semi-whorls. The essential oil was obtained from the herb in the flowering stage; the mass fraction of the essential oil was determined by the hydrodistillation method (Ginsberg's method) on the Clevenger apparatus. The study of the component composition of the essential oil (on the sample G1-21) was carried out by high-performance gas-liquid chromatography method. As a result, the length of the inflorescences was from 10.55 (G5-21) to 33.27 (G4-20) cm, the width of inflorescences from 3.52 (G3-22) to 6.51 (G4-20) cm, the number of whorls in inflorescences from 8.09 (G5-21) to 12.97 (G3-22) cm, number of flowers in semiwhorls from 10.58 (G4-20) to 39.38 (G1-21) depends on genotype. The essential oil content on dry weight was from 0.16 (G4-20) to 0.37 (G1-21) %. The prevailing components of the investigated essential oil of G1-21 were caryophyllene (17.46%) and germacrene D (17.80%); 1,8-cineol (8.83%), caryophyllene oxide (7.62%), germacrene D-epoxide (6.73 %), etc. found in less quantitative. Thus, the obtained data concerning the *N. grandiflora* plants showed differences in essential oil content and selected morphometrical parameters of plants depending on genotype. Also, the essential oil of N. grandiflora (G1-21) contains the principal chemicals that can be used in further pharmacological investigations, cosmetic, and food industries.

Keywords: giant catmint, morphometry, chromatogram, essential oil

Introduction

Medicinal and essential oil plant cultivation are promising, but, unfortunately, not sufficiently

developed branches of agriculture in Ukraine. The demand for raw materials significantly exceeds the supply on the domestic market, so most pharmaceutical enterprises are forced to import them

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from abroad (Vozhehova et al., 2022). Aromatic plants are renewable raw materials, that are used in food, pharmaceutical, and cosmetic industries for the creation of important products using these plants (Salamon, 2024). The well-known and useful aromatic and essential oil plants belong to the Lamiaceae family among which are representatives of the *Nepeta* L. genus which includes approximately 250 species.

Nepeta grandiflora M. Bieb. (giant catmint) is a perennial plant growing in Europe, Asia, Africa, the Balkans, etc. Plants from the *Nepeta* genus are well-known aromatic, medicinal, and ornamental plants with numerous pharmacological activities. *Nepeta* herbal tea is used for mental states such as hysteria, melancholy, asthenia, gastrointestinal and respiratory disorders, cystitis and prostate gland inflammation, dysmenorrhea, and syphilis, and externally for wound healing and against mastitis (Acimović et al., 2020).

The main metabolites identified in the essential oil of *Nepeta* spp. were nepetalactones, 1,8-cineole, α -pinene, α -terpineol, and caryophyllene oxide (Gkinis et al., 2003). The *Nepeta* spp. essential oil exhibited repellent activity due to the content of germacrene D and geranyl acetate, especially *N. grandiflora* and *N. clarkei* Hook (Birkett et al., 2010).

The extracts of *Nepeta* spp. plant raw demonstrated activity on the central nervous system, antifungal, antiviral, antinociceptive, analgesic, phytotoxic, spasmolytic, bronchodilatory, anti-atherosclerotic (Formisano and Rigano, 2011), cytotoxic, anti-tumour (Emami et al., 2016), anti-inflammatory, antioxidant, antibacterial, and anticancer (Bhat et al., 2018) activities. The plant extracts of *N. meyeri* Benth. had a herbicidal effect (Kordali et al., 2015).

The water extracts of different *Nepeta* spp. characterized by the high total content of phenolic compounds (419.8–669 mg GAE.g⁻¹) compared with methanol (105.9–242.5 mg GAE.g⁻¹). Moreover, the water extracts of some *Nepeta* species are the source of biologically active compounds with numerous pharmacological activities (Dienaitė et al., 2018).

N. grandiflora is one of the most *Nepeta* species for which information is limited. The scientific reports mostly include *N. cataria* L., *N. racemosa* Lam., *N. meyeri* Benth., *N. mussinii* Spreng. ex Henckel, etc. This study aimed to evaluate the biological features of plants and the biochemical content of the essential oil of *N. grandiflora* genotypes grown in the South of Ukraine.

Materials and methodology

Plant material and region characteristics

The study was conducted in the Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine (experimental collection of aromatic and essential oil plants in the Kherson Oblast, v. Plodove) in 2020–2022. The Nepeta grandiflora (Figure 1) genotypes were investigated: G1-21, G2-20, G3-22, G4-20, and G5-21 in the flowering stage.



Figure 1 *Nepeta grandiflora* M. Bieb. 3-years plants in the flowering stage

The main characteristic of the research region is a moderately continental climate, short-term springs, long-term dry and hot summers, mild winters, active temperature sum (>10 °C) is 3,200–3,400°C the annual rate of precipitation amount is 340-400 mm, soils are sandy and medium loam chernozem with 2.25% of humus and the neutral reaction of the soil solution (pH = 6.6-6.8). The minimal moisture content of arable soil ranged from 24.4 to 28.8% (Dudchenko et al., 2020). The sample *N. grandiflora* 2-08 was introduced in 2009 from Nikita Botanical Garden (Crimea, Ukraine) to the Kherson Oblast. The many years of investigation allowed us to collect seeds and get the genotypes described in this study.

Morphometrical parameters and selected measurement

The morphometric parameters of plants were used in this study: height of the plant (in cm), the diameter of the plant (in cm), length and width of leaves (cm), length and diameter of inflorescence (in cm), length of calix (in cm), corolla length (in cm). Also, was conducted seed sowing in the late-autumn period (before winter) and spring sowing and registered sprouts for calculating field seed germination (in %). The start of every phenological phase (vegetation, budding, flowering, and fruiting) was fixed. The mass of 1000 seeds was measured. In the genotype, plants were measured following parameters: length and width of inflorescences, the number of whorls in the inflorescences, the number of flowers in the semi-whorls. Also, were described inflorescences shape.

The essential oil content determination

The essential oil was obtained from the herb (aerial mass of plants) in the flowering stage; the mass fraction of the essential oil was determined by the hydrodistillation method (Ginsberg's method) on the Clevenger apparatus, based on the absolutely dry mass of the plant material (Elyemni et al., 2019). Fixed weight (approximately 100 g) put in the flask with 300–400 ml of water. The content of the obtained oil is calculated in percentages.

The essential oil composition determination

The study of the component composition of the essential oil was carried out by the method of highperformance gas-liquid chromatography on an Agilent Technology 6890N chromatograph. Chromatographic column quartz, capillary HP 5MS Evaporator temperature 250°C. The carrier gas is helium. The speed of the carrier gas is 1 ml/min. Sample introduction with a flow split of 1/50. Thermos temperature 50°C With programming 3°/min up to 220°. The temperature of the detector and evaporator is 250°C. The components of essential oils were identified based on the results of the search for the obtained in the mass spectra process of chromatography of the chemicals included in the

studied mixtures with the data of the NIST02 mass spectrum library. Component retention indices were calculated based on the results of control analyses of essential oils with a set of normal alkanes.

Statistical analysis

The results are expressed as mean values of three replications \pm standard deviation (SD). Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test (p <0.05).

Results and discussions

In the South of Ukraine, introductory trials are held in many aromatic and essential oil plants such as Artemisia L., Salvia L., Thymus L., etc. (Svydenko et al., 2022; Vergun et al., 2023). As reported in some studies, N. grandiflora grows in Central Europe and Central Asia (Said-Al Ahl et al., 2015) and also in Ukraine (Skybitska and Mohyliak, 2013). In Kherson Oblast, N. grandiflora was sown in late autumn (from the end of October to the beginning of November). With such sowing, seedlings appear in the second decade of April. The field seed germination was in the range of 70–75% with an average of 73.25% (Table 1). The growth and development of the aerial part of *N. grandiflora* begins with the appearance of small cotyledon leaves above the soil surface. After 15 days, the first real leaves appeared on the stairs. Self-seeding can also be observed in spring. With spring sowing (March-April), seedlings appear after 25-30 days, but the field seed germination (spring sowing) is less (12-15%) with an average of 14.09 %, and the seedlings are unfriendly.

Table 1Some biological, morphological, and morphometrical features of Nepeta grandiflora Bieb. plants in the South
Ukraine

Parameter	Result of measurement/observation
Field seed germination (late-autumn sowing), %	73.25 ±2.41
Field seed germination (spring sowing), %	14.09 ±0.55
Plant height of the first year, cm	71.11 ±3.76
Plant height of second year, cm	126.42 ±5.81
Plant diameter of second year, cm	118.13 ±6.78
Stem diameter, cm	1.18 ±0.06
Leaf length (flowering stage), cm	4.33 ±0.27
Leaf width (flowering stage), cm	2.77 ±0.12
Calix length, cm	0.81 ± 0.03
Corolla length, cm	1.13 ±0.05
Inflorescence length, cm	25.46 ±2.57
Inflorescence width, cm	4.89 ±0.32
Mass of 1000 seeds, g	0.32 ± 0.04
Start of spring vegetation	1 st , 2 nd week of March
Start of budding stage	3 rd week of May
Start of mass flowering	2 nd week of June
Start of fruiting	1 st , 2 nd week of July



Figure 2Inflorescences of Nepeta grandiflora Bieb. genotypes.

According to Svitelsky et al. (2018), the plant height of *N. cataria* in Polissya was 97 cm (1st year) and 107.8 cm (2nd year), leaf length was 3.8 and 4.8 cm, respectively, and leaf width was 2.4 and 3.1 cm, respectively.

We selected 5 genotypes of *N. grandiflora* based on a complex of economically valuable traits. The plants differed both in terms of morphological features (the size and shape of inflorescences, the number of whorls in inflorescences, and flowers in whorls) (Figure 2) and the content of essential oil.

The main morphometrical parameters of inflorescences of *N. grandiflora* genotypes are represented in Table 2. The length of the inflorescences was from 10.55 (G5-21) to 33.27 (G4-20) cm, the width of inflorescences from 3.52 (G3-22) to 6.51 (G4-20) cm, the number of whorls in inflorescences from 8.09 (G5-21) to 12.97 (G3-22) cm, number of flowers in semi-whorls from 10.58 (G4-20) to 39.38 (G1-21) depends on genotype.

One of the most important raw of the aromatic plants is essential oil rich in biologically active components (León-Méndez et al., 2019; Truchan et al., 2019). As a result, the essential oil obtained from the *N. grandiflora* plant raw in the conditions of introduction (Kherson Oblast) is characterized by a little yellowish and a pleasant floral aroma. It was established that essential oil content increased from the budding stage till the mass flowering stage, and depended on the plant genotype (Figure 3). The yield of *N. grandiflora* essential oil on dry and fresh weight was 0.16–0.37 % and 0.05–0.12 %, depending on genotypes.

According to Coltun et al. (2023), the content of essential oil in *N. grandiflora* herb from Moldova was from 0.31 to 0.42 %, which was higher than this study's results. Among the main groups of essential oil compounds are monoterpenes, diterpenes, triterpenes, sesquiterpenes (Formisano and Rigano, 2011), monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, etc. (Emami et al., 2016). The study of *N. kotschyi* Boiss. the essential oil showed a decrease in its content during vegetation from 2.48 % (vegetation) to 0.80–0.82 % (flowering-fruiting) (Habibvash et al., 2020).

Parameter	G1-21	G2-20	G3-22	G4-20	G5-21
Length of inflorescence, cm	27.11 ±0.25	29.13 ±0.45	25.04 ±1.12	33.27	10.55 ±0.87
				±1.43	
Width of inflorescence, cm	5.51 ±0.23	5.5 ± 0.41	3.52 ± 0.22	6.51 ± 0.46	4.03 ±0.11
The number of whorls in	11.09 ±1.12	12.43 ±0.36	12.97 ±0.54	10.32	8.09 ±0.21
inflorescence				±0.56	
Number of flowers in semi-	39.38 ±1.23	35.12 ±0.67	17.57 ±0.67	10.58	13.89 ±0.23
whorls				±0.34	
Shape of inflorescence	Cylindrical and	Cylindrical and	Spica-shaped, not	Elongated,	Short cylindrical
	dense	dense	fully dense	loose	dense

Table 2 Morphometrical characteristic of Nepeta grandiflora M. Bieb. genoty	pes
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Figure 3 The mass fraction of essential oil of *Nepeta grandiflora* M. Bieb. herb depending on genotype. Note: FM – fresh mass, DM – dry mass.

The study of essential oil qualitative and quantitative content is very important for the raw evaluation of aromatic and essential oil plants. Essential oil is a mixture of volatiles obtained by distillation or cold expression and includes mono and sesquiterpenes, etc. (Cagliero et al., 2021). Numerous plant species from Lamiaceae are used in folk and traditional medicines of many countries because of the specific content of essential oils that can be useful in pharmacology, cosmetology, and the food industry (Rattan, 2023). This study represented the results of essential oil investigation by the high-performance gas-liquid chromatography of N. grandiflora (G1-21) grown in the Kherson Oblast conditions, which was selected due to high productivity and essential oil yield (Figure 4). There were identified 52 components of investigated essential oil.

Twenty-nine components were identified in the essential oil of G1-21. The prevailing components of the investigated essential oil were caryophyllene (17.46%) and germacrene D (17.80%). 1,8-cineol (8.83%), caryophyllene oxide (7.62%), germacrene D-epoxide (6.73%), etc., were found in less quantity (Table 3).

It is a well-known fact, that *Nepeta* spp. essential oil contains nepetalactone isomers, which is the principal compound for these plants. Hadjieva et al. (1996) determined in the essential oil of *N. grandiflora* nepetalactone 4a β ,7 α ,7a α (41%) and nepetalactone 4a β ,7 α ,7a β (2.4%), while in the present study wasn't detected this compound. The high content of 4a α ,7 α ,7a α -nepetalactone was detected in the essential oil of *N. racemosa* (64.9%) (Rustaiyan et al., 2000), 4a β ,7 α ,7a β -nepetalactone in *N. cataria* (53.87–58%) (Zomorodian et al., 2013; Ashrafi et al., 2019), 4a α ,7 α ,7a β -nepetalactone in *N. meyeri* (83.7%)

(Kordali et al., 2015). According to Salehi et al. (2018), the essential oil of *N. grandiflora* relates to the *Nepeta* spp. group with a medium content of nepetalactones. Birkett et al. (2010) detected that essential oils of *N*. grandiflora and N. clarkei had very low nepetalactone content, while sesquiterpene compounds prevailed. According to this study, germacrene D (54.53%), caryophyllene (25.48%), and 1,8-cineole (4.21%) were the dominant identified components (Birkett et al., 2010). The study of N. grandiflora essential oil grown in Egypt demonstrated the most content of pcymene (43.46%), c-terpinene (18.58%), carvacrol (12.95%), o-cymene (10.99%). Other important components of this essential oil were transcaryophyllene (2.04%), α -thujene (1.69%), and α bisabolene (1.57%) (Said-Al Ahl, et al. 2015). Similar results were represented by Mohamed et al. (2018) for N. grandiflora (45.13% of p-cymene and 16.45% of cterpinene).

The content of different biochemical compounds in plant raw of N. nuda such as sesquiterpenes, according to Aćimović et al. (2022), depended on the vegetation period and the difference between study years was 2.0-2.5 times. According to Acimović et al. (2022), 1,8cineole (46.1%), germacrene D (9.9%), caryophyllene oxide (8.1%), trans-caryophyllene (5.0%) were the most dominant components of N. nuda essential oil. As reported Coltun et al. (2023), the germacrene D (28%), eucalyptol (27.1%), and β -pinene (5.6%) were determined in *N. grandiflora* from the Moldova region as prevailed. Also, in that study found 4.1% of spathulenol, and 3.7% of sabinene, while in our investigation sabinene content was 0.31%. A similar content of 1.8-cineole (8.83%) was obtained in the essential oil of N. racemosa by Dabiri and Safidkon (2003) compared with our results.

Abundance



Figure 4 Chromatogram of Nepeta grandiflora Bieb. essential oil (Genotype 1-21)

Component	Content	Component	Content index,
	index, %	-	%
Sabinene	0.31	Metylcarvacrol	0.38
β-pinene	0.25	Thymol	3.88
Octanone-3	0.17	Carvacrol	0.23
Myrcene	0.15	α-copaene	0.55
Cimene	0.73	β-bourbonene	1.69
Limonene	0.51	β-elemene	0.92
1,8-cineol	8.83	Caryophyllene	17.46
trans-Sabinene hydrate	0.14	Humulene	1.13
Linalool	0.29	Germacrene D	17.80
trans-epoxy ocimene	0.11	Eremophilene	1.01
Menthol	0.31	δ-cadinene	0.28
Terpinen-4-ol	0.22	Caryophyllene oxide	7.62
		2-methylene-6,8,8-trimethyl-	
		tricyclo[5.2.2.0(1,6)]	
α-terpineol	0.13	undecane-3-ol	1.48
<i>cis</i> -2,6-dimetyl-3,5,7-oktatrien-2-ol	0.35	Germacrene D– epoxide	6.73
trans-2.6-dimetyl-3.5.7-octatrien-2-ol	0.49		

Table 3 The main components of Nepeta grandiflora Bieb. essential oil (Genotype 1-21)

Conclusions

Thus, as a result of the introduction of *N. grandiflora* in the Kherson Oblast (Ukraine), these plants can be recommended for further cultivation in this region. An extreme string message is an answer to a question. The current generation of *Nepeta grandiflora* is not homogeneous and contains genotypes that differ in both morphometric and economically valuable traits. The mass fraction of essential oil of investigated genotypes varied from 0.16 to 0.37%. The study of the essential oil composition of the most productive genotype G1-21 showed a prevailing presence of caryophyllene and germacrene D and an absence of nepetalactone. These results can be useful for further pharmacological, biochemical, and breeding studies with this plant species.

Conflict of interest

The authors declare no conflict of interest to declare.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

The authors are thankful for the support of this research through the Bilateral Scholarship Program and International Visegrad Fund.

Acknowledgments

The publication was prepared with the active participation of researchers involved in the International Network AgroBio*Net* of the Institutions and researchers for the realization of the research, education, and development program «Agrobiodiversity for improving nutrition, health, and life quality».

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