

**RESEARCH ARTICLE**

## Component Composition and Antimicrobial Activity of *Dracocephalum nutans* L. Essential Oil

Gayane Atazhanova<sup>1</sup>, Assel Sabiyeva<sup>1</sup>, Saule Akhmetova<sup>1</sup>, Marlen Smagulov<sup>2</sup>,  
Aigul Medeshova<sup>1</sup>, Sholpan Sarsembayeva<sup>1</sup>, Aiman Sarsembayeva<sup>2</sup>, Ulpan Aldabayeva<sup>1</sup>,  
Gulnissa Kurmantayeva<sup>1</sup>

<sup>1</sup>NCJSC Karaganda Medical University, Gogol Str. 40., 100000, Karaganda, Kazakhstan

<sup>2</sup>NCJSC "Karaganda University named after E.A. Buketov, Universitetskaya Str 28, block 3., 100028,  
Karaganda, Kazakhstan

\*Corresponding Author E-mail: [aseka9520@mail.ru](mailto:aseka9520@mail.ru)

### ABSTRACT:

Currently, the arsenal of the pharmaceutical market has significantly expanded with highly effective drugs of plant origin. More than 1000 essential-oil-bearing plants grow in the flora of Kazakhstan. Of great interest are some species from the families Lamiaceae, Apiaceae, Asteraceae, which have not been studied at all before, or for which there is only brief information about the chemical composition and biological properties. In this respect, the Lamiaceae L. family, which is one of the leading in the flora of Kazakhstan, is of interest. So, on the territory of the republic in this family, there are 233 species, united in 45 genera. Among them, the most famous are plants of the genus *Dracocephalum*, which are a rich and very common source of essential oils. The aim of the study was to investigate volatile compounds from the aerial parts of *Dracocephalum nutans* L. of the genus *Dracocephalum* of the family Lamiaceae grown wild in Central Kazakhstan. *D. nutans* – perennial with a beautiful purple flower. **Materials and methods.** The aim of the study was to study volatile compounds from the aerial parts of *Dracocephalum nutans* L. of the genus *Dracocephalum*, Lamiaceae family grown wild in Central Kazakhstan. The oils were obtained using Clevenger apparatus, their composition was evaluated by means of gas chromatography/mass spectrometry (GC/MS). The antibacterial activity of *D.nutans* essential oil sample was assessed by the diameter of the growth inhibition zones of the test strains (mm). **Results and Discussion.** The main natural components of *D.nutans* essential oil were 1.8-cineol (34%),  $\alpha$ -pinene (6.7%),  $\beta$ -pinene (5.2%),  $\beta$ -mircene (5.3%),  $\alpha$ -thujone (8.0%) and  $\beta$ -thujone (5.3%). The results obtained showed differences in the composition of essential oils obtained from already studied *D. nutans*. 1.8-cineol was also found in *D. nutans* as one of the major component. **Conclusion.** As a result of a study on antimicrobial activity, it was established for the first time that a sample of *D. nutans* essential oil exhibited pronounced antimicrobial activity against strains of gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and weak antimicrobial activity against gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*.

**KEYWORDS:** *Dracocephalum nutans* L.; essential oil; gram-positive bacteria; antibacterial activity; gram-negative bacteria; GC/MS.

### INTRODUCTION:

Currently, a huge number of microorganisms, primarily intrahospital strains, pose a threat to human life and health. The main reason for its formation is the massive use of antibacterial preparations, which in some cases leads to undesirable consequences: dysbiosis, anaphylactic shock. In this regard, a search is underway for new preparations that, on the one hand, have antimicrobial activity with a mechanism different from the usual antibiotics, and, on the other hand, have no side effects<sup>1,2</sup>.

In the modern world, the problem of bacterial resistance to antibiotics is becoming increasingly important. Essential oils and their components are of significant scientific and practical interest due to their antimicrobial activity against strains and microorganisms resistant to antibiotics. And in this regard, there is an increased interest in essential-oil-bearing plants with antimicrobial activity<sup>3,4</sup>. These plants include *Dracocephalum nutans* L. (*D.nutans*).

The genus *Dracocephalum* L. belongs to the family *Lamiaceae* Lindl. (*Labiatae*), whose plants are of interest as sources of medicinal preparations<sup>5-12</sup>. *Dracocephalum nutans* L. contains a complex of biologically active substances and is used in the medicine of the peoples of Southeast Asia in the treatment of inflammation of the kidneys and gastrointestinal diseases such as hepatitis, gastritis, etc.

## MATERIALS AND METHODS:

### Raw materials:

The collection of plant raw material was carried out in the places of natural growth of *D.nutans* in Central Kazakhstan: in the Karaganda region, in the vicinity of the village of Karkaralinsk (52°56'N: 70°18'E). Samples for the study were collected on May 20, 2020 at the full flowering stage (leaves, stems and flowers) (Fig. 1). The projective cover of *D.nutans* did not exceed 10–15%. Identification and documentation (sampling certificates) of plant species were carried out by Professor M. Ishmuratova using “Flora of Kazakhstan” as a plant guide<sup>13-18</sup>.



Figure 1. General view of *Dracocephalum nutans* L.

### Isolation of the essential oil:

The essential oil of the fresh samples (aerial parts: leaves, stems and flowers) (100g) was isolated by hydrodistillation for 3hours with the use of the Clevenger-type apparatus. Hydrodistillations were repeated 3 times.

### Qualitative and quantitative analyses:

To study the composition of the essential oil, a chromatograph-mass spectrometry method was used with the help of an Agilent Technologies 7890A gas chromatograph with a quadrupole mass spectrometer MSD 5975 C as a detector. The capillary column HP-5MS had a size of 30m x 0.25mm (film thickness 0.25 μm). Evaporator temperature was 230<sup>0</sup>C. The gas chromatographic column was kept at 40<sup>0</sup>C for 10 minutes; with temperature programming up to 240m/z at a temperature change rate of 2<sup>0</sup>C/min, and then held in isothermal mode for 20minutes. Sample injection mode was flow division 100:1. Sample volume was 0.2μl. The conditions for recording mass spectra are 70eV, the mass range is 10-360m/z. For data processing, the MSD ChemStation software, supplied by Agilent Technologies, was used in combination with AMDIS 32 and NIST 2017.

The percent contents of the constituents were calculated automatically using peak areas in the total ion chromatogram without using correction factors (Figure 2, Table 2). Constituents were identified using mass spectra, retention times, and the Wiley GC/MS library. The results were also confirmed by the comparison of the compounds with their relative retention indices in the literature. To obtain the retention indices there was used a standard solution of n-alkanes (C8-C24).

The study of the antimicrobial activity of *D.nutans* essential oil was carried out in relation to strains of gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and gram-negative strains of *Escherihia coli*, *Pseudomonas aeruginosa* by diffusion into agar (wells). Reference drugs - benzylpenicillin sodium salt and eucalyptus oil for bacteria.

The cultures were grown in a liquid medium pH 7.3±0.2 at a temperature of 30 to 35<sup>0</sup>C for 18-20hours. The cultures were diluted 1:1000 in a sterile 0.9% isotonic sodium chloride solution, 1ml was added to cups with appropriate elective nutrient media for the studied test strains, and sown according to the “solid lawn” method. After drying, wells 6.0mm in size were formed on the surface of the agar, into which solutions of the test sample, benzylpenicillin sodium salt 10μl each and eucalyptus oil 1μg each were added, 96% ethyl alcohol was used in control in equivolume amounts. Thus, the studied samples were tested in the amount of 1μg, and the reference drug in the amount of 1mg. The inoculations were incubated at 37<sup>0</sup>C, the growing cultures were counted after 24hours.

The antibacterial activity of *D.nutans* essential oil sample was assessed by the diameter of the growth inhibition zones of the test strains (mm). Each sample

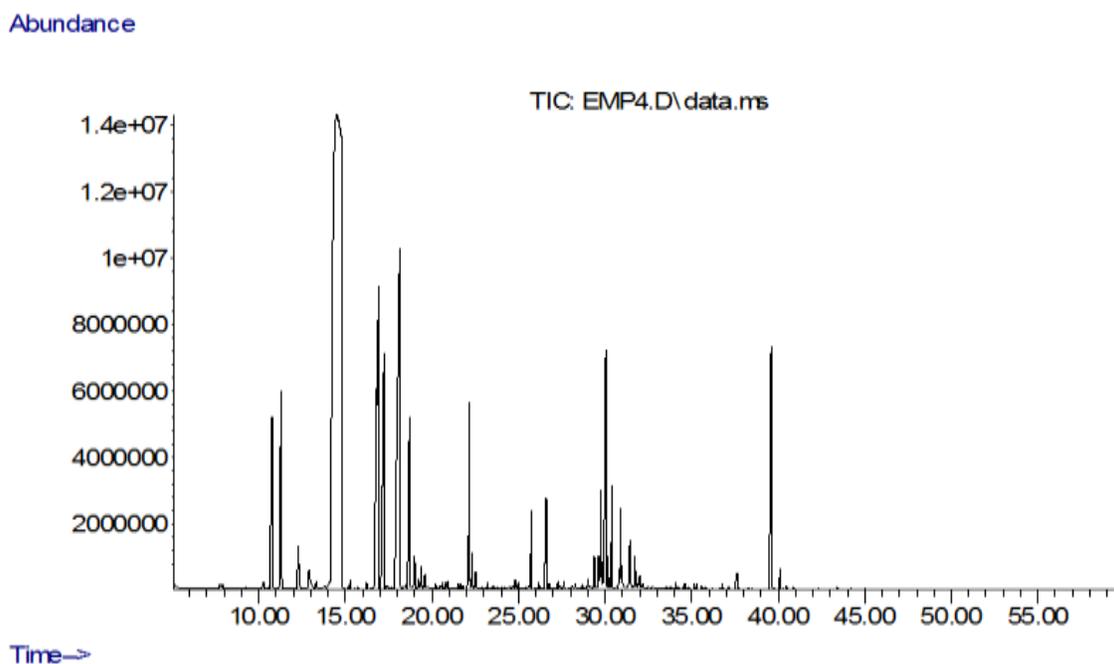
was tested in three parallel experiments. Statistical methods with the calculation of the arithmetic mean and processing was carried out using parametric statistics standard error.

**RESULTS AND DISCUSSION:**

**Table 1: Percentage composition of the essential oil from aerial parts of *Dracocephalum nutans* L.**

Sr. No.	Component	RT (min)	RI <sup>a</sup>	RI <sup>lit</sup> <sup>b</sup>	Content of components in sample (%)
1	(1R)-2,6,6-Trimethylbicyclo [3.1.1]hept-2-ene	10.75	929	930	6.6848
2	Camphene	11.28	952	952	5.2772
3	β-Pinene	12.27	979	975	5.7311
4	β-Myrcene	12.93	991	991	0.9378
5	Eucalyptol	14.48	1032	1031	47.0985
6	α-Thujone	16.7829	1103	1104	7.9601
7	β-Thujone	16.9199	1113	1114	5.3427
8	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1-α,4-β,5-α)]-	17.22	1114	1114	4.0520
9	(+)-2-Bornanone	17.97	1144	1144	2.1481
10	endo-Borneol	18.7093	1167	1168	2.5264
11	Terpinen-4-ol	18.9979	1177	1177	0.2939
12	Benzenemethanol, α, α,4-trimethyl-	19.2288	1183	1186	0.1296
13	α-Eerpineol	19.3876	1189	1191	0.1827
14	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	22.1655	1284	1287	1.9532
15	Phenol, 2-ethyl-4,5-dimethyl-	22.3747	1305	1305	0.2034
16	(-)-β-Bourbonene	24.8135	1384	1387	0.1033
17	Caryophyllene	25.7370	1419	1422	0.6363
18	Humulene	26.6029	1454	1456	0.7502
19	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1-α,3-α,β-4-α,7-β)]-	30.0446	1473	1476	3.3409
20	Ledol	30.2394	1565	1567	1.2033
21	α-Bisabolol	32.0071	1684	1688	0.8034
22	1-Naphthalenepropanol, α-ethenyldecahydro-α,5,5,8a-tetramethyl-2-methylene-, [1S-[1α(R*),4αβ,8αα]]-	39.58	2056	2058	2.6035
	Total				100.0

Note: <sup>a</sup> Retention Indices on HP-5ms column;  
<sup>b</sup> Retention Indices in literature (Adams 2017)



**Figure 2: Gas chromatogram of the essential oil of the aerial parts of *D.nutans***

**Table 2. The main components of *D.nutans* essential oil from different places of growt**

Species	Place of growth	Source	Main components	References
<i>D.nutans</i> L.	The plant for study was collected in the territory of East Kazakstan, Katon-Karagay district, the neighborhood of the village Enbek, the mountain Shagyl	essential oil was isolated from air-dried and ground aerial parts and distilled with water for 3 h using a Clevenger-type apparatus	<i>cis, cis</i> -nepetalactone – 35,0%, germacrene D – 6,3%, $\beta$ -cyclocitral – 4,0%, $\beta$ -bourbonene – 3,1% and <i>cis, trans</i> -nepetalactone – 2,9%.	Ye.M. Suleimen et al
	Altay region	The oils were isolated by water–distillation for 4 hrs and then dried over anhydrous sodium sulphate.	The major volatile oils of 1H-Cycloprop[e] Azulen-7-ol, decahydro-1,1,7- trimethyl- 4- methylene -(14.23%), Caryophyllene oxide (11.30%), 1,6-Octadien- 3- ol, 3,7 -dimethyl-(7.69%), (-) - myrtenyl acetate (5.67%), Naphthalene, 1,2,3,4,4A,5,6,8A-octahydro- 7- methyl-4 -methylene (5.29%), (-) -spathulenol (4.76%), Benzene, 1- Methoxy- 4-(1- propenyl)-(4.73%), 4 ah - cycloprop[e]azulen – 4A- ol, decahydro- 1, 1, 4, 7- tetra-[ethenyl]- (4.65%), Germacrene D (4.42%), (E)- 3(10)- caren-4-ol (4.23%), Bicyclo[7.2.0]undec -4-ene, 4,11,11- trimethyl-8-methulene -(3.67%), $\alpha$ - Caryophyllene (3.60%), 12-oxabicyclo [9.1.0]dodeca-3,7- diene, 1,5,5,8- tetramethyl (2.13%).	Baiseitova A.M. et al.
	Kashmir region of Himalaya	essential oil was isolated from air-dried and ground aerial parts and distilled with water for 3 h using a Clevenger-type apparatus	pinocamphone (56,4%), $\beta$ -pinene (12,7%), isopinocampnone (4,3%), $\alpha$ -phellandrene (4,6%) and isopinocampheol(3,7%)	Misra et al

**Table 3. - Antibacterial activity of the test sample against gram-positive and gram-negative bacteria (inhibition zone measured in mm)**

<i>mm</i> Sample cypher	<i>Staphylococcus aureus</i> ATCC 6538	<i>Bacillus subtilis</i> ATCC 6633	<i>Escherichia coli</i> ATCC 25922	<i>Pseudomonas aeruginosa</i> ATCC 27853
EZP (essential oil)	25±1	26±1	14±1	10±1
Benzylpenicillin sodium salt	16 ± 0,1	14 ± 0,1	15 ± 0,1	12±1
Eucalyptus oil	-	14 ± 0,1	-	-
Ethanol (96%)	10 ± 0,1	11± 0,1	10 ± 0,1	10 ± 0,1

Note: “-“ - there was no growth retardation zone, the diameters of growth retardation zones were less than 10 mm and continuous growth in the dish was assessed as the absence of antimicrobial activity, 10-15 mm - weak activity, 15-20 mm - moderately pronounced activity, over 20 mm – pronounced.

## CONCLUSION:

As a result of a study on antimicrobial activity, it was established that a sample of *D. nutans* L. essential oil exhibited pronounced antimicrobial activity against strains of gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and weak antimicrobial activity against gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*.

**Thus, *D. nutans* essential oil has the following benefits:**

- Pronounced antimicrobial activity against strains of gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*;
- Weak antimicrobial activity against gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*.

## CONFLICT OF INTEREST:

The authors declare no conflict of interest.

## REFERENCES:

1. Shruthi Chandrasekaran, Geetha. R.V. Antibacterial Activity of the Three Essential Oils on Oral Pathogens- An In-vitro Study. Research J. Pharm. and Tech. 7(10): Oct. 2014 Page 1128-1129
2. Sabiyeva A., Ishmuratova M. Yu., Atazhanova G. A., Smagulov M. K., Zhuravel I. A. Histochemical Analysis of Aerial part of *Dracocephalum ruyschiana* L. and *Dracocephalum nutans* L. growing in the Territory of Central Kazakhstan. Research Journal of Pharmacy and Technology. 2022; 15(9):3831-5. doi: 10.52711/0974-360X.2022.00642
3. Pavlov, N.V., editor. 1963. Flora of Kazakhstan. Vol. 6. Alma-Ata: Publishing House of the Academy of Sciences of Kazakh SSR. Russian.
4. Flora of USSR: in 30 volumes / Editor Komarov, V.L. (1954) - M.; L.: Publishing House of the Academy of Sciences of the USSR. - Vol. 20
5. Gubanov, I.A. *Dracocephalum nutans* L. — Illustrated guide to plants of Central Russia: in 3 volumes / Gubanov, I.A., Kiseleva, K.V., Novikov, V.S., Tikhomirov, V.N. - M.: Scientific Publications Association KMK: Institute for Technological Research, 2004. — Vol. 3: Magnoliophyta (Dicotyledoneae: Monopetalae). 116. 520 p. — ISBN 5-87317-163-7.
6. Gammerman, A.F., Shupinskiy, M.D. (1937). Preliminary chemical study of medicinal raw materials of Tibetan medicine,

- collected by the Transbaikal expedition of VIEM // Farmatsiya i Farmakologiya. 4:20-21.
7. Levaya , Y. K.; Erkenuly, Z. M. .; Abdulkhakimovna, A. G. .; Boltabaevna , A. S. . Evaluation of Antibacterial Activity of Salvia Stepposa Extracts Isolated Using Microwave Extraction, Growing Wild in Kazakhstan. Trends Sci 2022, 19, 3217.Suleimen, Ye.M., Myrzagaliyeva, A.B., Ibatayev, Zh.A., Iskakova, Zh.B., Samarkhanov, T.N., Medeubayeva, B.Z. (2016).
  8. Component composition and biological activity of essential oils of genus Dracocephalum L. // Khimiya rastitel'nogo syriya. №4. P. 83-88.
  9. Misra, L.N., Shawl, A.S., Raina, V.K. (1988). Planta Med., 54:165-166.
  10. Baiseitova, A.M., Aisa, H., Jeni, S.J. (2015). International Journal of Biology and Chemistry. 8:90-97.
  11. T. Thilagavathi, G. Kathiravan. Phytochemical Analysis and Antimicrobial Activity of Ethonolic Leaf Extract of Ficus racemosa Linn. Research J. Pharm. and Tech. 2017; 10(2): 537-540. doi: 10.5958/0974-360X.2017.00107.X
  12. G. Muthu Bhupathi, K. Padmalatha, Akkiraju Anusha, Abdul Rameeza, Makina Geethika Sravanthi, Sunnam Praneetha. Synthesis, Characterization and Antimicrobial Activity of Acetanilide Derivatives by using Aromatic Aldehydes and Sulphonamide Derivatives. Research J. Pharm. and Tech 2016; 9(11): 1846-1854. doi: 10.5958/0974-360X.2016.00377.2
  13. Basanti Majhi, Kunja Bihari Satapathy, Sagar Kumar Mishra. Antimicrobial activity of Averrhoa carambola L. leaf extract and its Phytochemical Analysis. Research J. Pharm. and Tech. 2019; 12(3): 1219-1224. doi: 10.5958/0974-360X.2019.00202.6
  14. Haitham Qaralleh, Khaled M Khleifat, Ali M Khlaifat, Muhamad Al-limoun, Nafe M Al-Tawarah, Amir Menwer Alhroob, Ahmad B Alsaudi. Chemical Composition, Antioxidant and Inhibitory Effect of Cupressus sempervirens Essential Oils and Methanolic Extract on Beta-lactamase producing Isolates. Research Journal of Pharmacy and Technology. 2021; 14(9):4673-9. doi: 10.52711/0974-360X.2021.00812
  15. Monica Joicy, C, Sivaraj, C, Arumugam, P. In-vitro Antioxidant, Antidiabetic, Antibacterial and Cytotoxic Activities of Essential Oil extracted from Flowers of Illicium verum L. Research Journal of Pharmacy and Technology. 2021; 14(5):2452-8. doi: 10.52711/0974-360X.2021.00431
  16. Riham Omar Bakr, Soumaya Saad Zaghoul, Reham Ibrahim Amer, Dalia Abd Elaty Mostafa, Mahitab Helmy El Bishbishy. Formulation, Characterization and Antimicrobial efficacy of Aegle marmelos Essential oil nanogel. Research Journal of Pharmacy and Technology. 2021; 14(7):3662-8. doi: 10.52711/0974-360X.2021.00633
  17. Sabiyeva A., Ishmuratova M. Yu., Atazhanova G. A., Smagulov M. K., Kurmantayeva G. K., Ashirbekova B. B., Taiken A. A.. Anatomical study of Dracocephalum ruyschiana L. and Dracocephalum nutans L. Research Journal of Pharmacy and Technology 2023; 16(3):1193-8. doi: 10.52711/0974-360X.2023.00198
  18. Amantayeva M, Kozhanova K, Kadyrbayeva G, Medeshova A, Tulebayev Y, Zhandabayeva M, Yeleken G, Allambergenova Z, Czigle S. Macroscopical, Microscopical and Histochemical Analysis of Eryngium karatavicum Iljin Growing on the Territory of South Kazakhstan. Plants (Basel). 2023 Jul 21;12(14):2714. doi: 10.3390/plants12142714. PMID: 37514327; PMCID: PMC10384362.