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COMPOSITION AND BIOLOGICAL ACTIVITY OF ESSENTIAL OIL FROM PLANTS ENDEMIC TO KAZAKHSTAN

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The chemical compositions of essential oils from certain species of plants endemic to the flora of Kazakhstan were studied in the 1950s-1960s by Goryaev [1]. The use of GC methods available at that time limited the determination of the total composition of the isolated essential oils.

We used GC—MS to study the component compositions of essential oils from 18 species of plants endemic to the flora of Kazakhstan where the main components were 1,8-cineol, camphor, linalool, thymol, and α- and β-pinenes; a total of 23 compounds. Thus, 113 components, 88.6% of all observed, were identified in essential oil (1%) from the endemic species *Artemisia glabella* Kar. et Kir. (Karaganda Oblast′), which exhibits anti-inflammatory [2] and antifungal activity [3]. The main components were 1,8-cineol (10.0%), linalool (5.1%), and α-terpineol (4.2%). It is noteworthy for comparison that Bicchi et al. [4] used GC to determine 52 components in essential oil of *A. glabella*.

The chemical composition of essential oil from the new endemic plant *A. filatovae* A. N. Kuprijanov (0.1%, Pavlodar Oblast′), which was determined by GC—MS, numbered 117 components. The main components were *trans*-nerolidol (10.5%), 1,8-cineol (5.4%), and α-terpineol (4.4%). *A. filatovae* is morphologically similar to another endemic species *A. glabella*. Results from studies of the chemical compositions of essential oils from the two sage species indicate that they are different because the main component in *A. glabella* essential oil is 1,8-cineol; in *A. filatovae*, nerolidol. Therefore, these compounds can be considered distinguishing features of two similar sage species, i.e., their chemotaxonomic signatures.

An investigation of essential oil from *A. cina* Birg. (1.59%, South Kazakhstan Oblast′) identified 69 components, 95.4% of all those observed. The main ones were 1,8-cineol (46.0%), *p*-cymene (9.8%), and α-thujone (8.9%).

A total of 101 components, 93.0% of all those observed, among which the main ones were camphor (39.0%), camphene (9.3%), and 1,8-cineol (6.2%), were identified in essential oil of *A. leucodes* Schrenk (1.57%, Zhambyl Oblast′), which is endemic to Central Asia and southern Kazakhstan.

GC—MS was used to investigate essential oil from *A. kasakorum* (Krasch.) Pavlov. (0.1%, East Kazakhstan Oblast′), which is endemic to central and northern Kazakhstan. A total of 52 components was identified, i.e., 86.2% of all those observed. The main components were sabinylacetate (12.9%), *trans*-sabinol (4.6%), and 1,8-cineol (4.3%).

Essential oil was obtained in 1.0% yield as a colorless liquid with a pleasant aroma consisting of at least 110 components from the recently described endemic species *A. radicans* A. Kuprijanov (Karaganda Oblast′). The main components were camphor (24.6%), 1,8-cineol (20.5%), and borneol (5.4%).

The component composition of *A. quinqueloba* Trautv. essential oil (0.8%, Aktyubinsk Oblast′) numbered 54 compounds, the main ones of which were β-pinene (18.2%) and α-bisabolol (19.1%). β-Pinene is also the main component of essential oils from other species of the Asteraceae family, for example, in essential oil from the endemic species *A. albicerata* Krasch (1.15%; Aktyubinsk Oblast′; α-bisabolol, 15.2%; α-pinene, 7.5%) where its content reaches 20% and in essential oil from *A. tomentella* Trautv. Bull. Soc (0.8%, Karaganda Oblast′), 17%. A total of 111 components was observed in the last oil, among them α-pinene (20.4%) and β-myrcene (16.2%). Goryaev et al. established previously in *A. tomentella* oil a hydrocarbon content of 26% as a mixture of *l*-, α-, and β-pinenes, 12.45% bound alcohols as ethers, and 23.9% of free alcohols [5].

A total of 28 components or 64.8% was identified in essential oil from *Achillea ledebourii* Heimerl. (0.23%, East Kazakhstan Oblast′), the main ones of which were germacrene D (20.55%) and spatulenol (9.1).

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TABLE 1. Antibacterial Activity of Essential Oils in Microorganism Strains

Essential oil	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Candida albicans*	*Pseudomonas aeruginosa*	*Streptococcus agalactiae*
Artemisia glabella	24 ± 0.2	25 ± 0.2	20 ± 0.2	22 ± 0.2	23 ± 0.1	23 ± 0.1
A. cina	20 ± 0.1	18 ± 0.3	12 ± 0.1	17 ± 0.2	-	13 ±0.2
A. tomentalla	24 ± 0.1	20 ± 0.1	15 ± 0.3	15 ± 0.1	-	-
A. kasakorum	17 ± 0.2	19 ± 0.2	18 ± 0.1	13 ± 0.1	-	-
A. radicans	14 ± 0.2	20 ± 0.2	15 ± 0.1	14 ± 0.1	-	-
A. filatovae	20 ± 0.2	19 ± 0.2	18 ± 0.1	20 ± 0.1	21 ± 0.1	-
A. leucodes	17 ± 0.2	19 ± 0.2	18 ± 0.1	13 ± 0.1	-	-
Tanacetum scopulorum	18 ± 0.2	21 ± 0.1	14 ± 0.1	-	-	-
T. ulutavicum	16 ± 0.3	18 ± 0.2	14 ± 0.2	-	-	-
Thymus crebrifolius	21 ± 0.1	20 ± 0.1	15 ± 0.3	17 ± 0.3	-	-
Th. rasitatus	19 ± 0.1	17 ± 0.3	13 ± 0.1	13 ± 0.1	-	-
Th. roseus	24 ± 0.1	20 ± 0.1	15 ± 0.2	12 ± 0.1	-	-
Eucalyptus oil	-	25 ± 0.1	-	-	-	-
Gentamycin	26 ± 0.1	24 ± 0.1	23 ± 0.2	-	24.0 ± 0.1	23.0 ± 0.1
Nystatin	-	-	-	22 ± 0.1	-	-

Two species of endemic plants of the genus *Tanacetum*, *T. scopulorum* (Krasch.) Tzvel. collected in Karaganda Oblast′ (0.14%) and *T. ulutavicum* Tzvel. (0.1%), gave essential oils with the main components 1,8-cineol (25.5 and 15.6%) and camphor (14.3 and 20.2%), respectively.

Plants of the genus *Thymus* are widely distributed in Kazakhstan and number 27 species. We studied the component compositions of essential oils from endemic species of *Thymus* growing in Karaganda Oblast′. The main components from *T. lavrenkoanus* Klok. were *p*-cymene (32.2%), γ-terpinene (7.5%), and borneol (4.9%); from *T. roseus* Schipcz. (0.3%), *o*-cymene (14.1%), linalool (12.1%), *p*-menth-1-en-8-ol (10.6%), carvacrol (10.0%), terpinenol-4 (7.1%), and γ-terpinene (6.6%); from *T. rasitatus* Klok. (0.2%), thymol (23.6%), *p*-cymene (21.2%), γ-terpinene (11.1%), *p*-menth-1-en-8-ol (8.1%), and thymolmethylether (5.5%); from *T. crebrifolius* Klok. (0.9%), linalool (27.9%), *p*-cymene (12.9%), *p*-menth-1-en-8-ol (11.0%), and borneol (9.6%).

Essential oil (0.01%) from *Lagochilus diacanthophyllus* (Pall.) Benth, which is endemic to Central Asia and was collected in the Tarbagatai foothills (Zhambyl Oblast′), was a viscous yellowish liquid with a sharp unpleasant odor. A total of 71 components, 81.6% of those observed, was identified in the oil. The main components were α-pinene (8.7%) and dillapiol (3.1%).

The main components in essential oil from *Hyssopus macranthus* Boriss (0.8%) were 1,8-cineol (38.6%), α-pinene (8.1%), and linalool (7.3%) out of a total of 33 components.

Bioscreening by diffusion in agar of all studied essential oils showed distinct antimicrobial activity toward gram-positive strains (*Staphylococcus aureus*, *Bacillus subtilis*) and moderate activity toward gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*) (Table 1). The fungicidal activity toward the yeast *Candida albicans* was weak for most of the samples except essential oils of *T. crebrifolius*, *A. glabella*, and *A. filatovae*, which had no sustainable growth zones for the fungus.

The essential oils possessed different tuberculostatic activity toward various species of tuberculosis mycobacteria. Thus, essential oil of *A. filatovae* had tuberculostatic activity toward mycobacteria of bovine and avian species. Essential oils of *Artemia salina*, *A. glabella*, *A. filatovae*, and *Mentha piperita* exhibited activity toward human mycobacteria (strain H37RV) and a field strain.

Essential oils from 12 plant species showed cytotoxic activity toward nymphs of the marine crustacean *Artemia salina*; from *A. glabella*, *T. crebrifolius*, and *T. ulutavicum*, distinct cytotoxicity; and from *A. albicerata* and *A. quinqueloba*, slight activity.

Essential oil	*Toxic dose, LD_{50}, μg/mL*
Artemisia glabella	10.9
A. albicerata	27.7
A. cina	14.1
A. tomentalla	18.2
A. quinqueloba	23.6
A. filatovae	17.6
A. leucodes	15.4
Tanacetum scopulorum	18.5
T. ulutavicum	12.4
Thymus crebrifolius	8.0
Th. rasitatus	15.6
Th. roseus	10.1
Sesquiterpene lactone arglabin	24.0

Thus, essential oils of the studied plant species endemic to Kazakhstan are potential sources of new biologically active compounds, mainly antimicrobial, cytotoxic, and antituberculosis agents.

Essential oils were prepared by steam distillation in a Clevenger apparatus. The distillation time was 3 h. Yields of essential oils were calculated based on air-dried mass of raw material.

The qualitative compositions of essential oils were analyzed by GC—MS on an Agilent 6890N instrument with an Agilent 5973N mass spectrometric detector. We used a DB-XLB FSC capillary quartz column (30 m × 0.25 mm) with He carrier gas. Qualitative analysis was based on comparison of retention times and full mass spectra with corresponding data of standard oil components and pure compounds, if they existed, and with data in mass spectra libraries (Wiley 7th Ed., 390,000 spectra, and NIST 02, 175,000 compounds).

Antimicrobial activity of samples was studied against strains of gram-positive bacteria *S. aureus*, *B. subtilis*, and *Streptococcus agalactiae*; gram-negative strains *E. coli* and *P. aeruginosa*; and yeast *C. albicans* by diffusion in agar (wells). Strains were cultivated in meat-peptone broth liquid medium, pH 7.3 ± 0.2, 30-35°C, 18-20 h. Cultures were diluted 1:1000 with sterile normal isotonic saline (0.9%), placed in dishes (1 mL) with the corresponding selected nutrient media for the studied test strains, and innoculated by the continuous field method. Studied samples were dissolved in ethanol (96%) at a concentration of 1 mg/mL. Innoculations were incubated at 37°C. Growing cultures were observed every 24 h.

Antituberculosis activity was determined using three standard strains of mycobacterium Bovis-8 (bovine), Avium-780 (avian), H37RV (human), and a field strain isolated from sputum of patients with destructive forms of tuberculosis. Solutions of essential oils were prepared with concentrations of 300 μg/mL. Then suspensions of mycobacteria (0.2 mL) were mixed with essential oils (0.3 mL), mixed with NaCl solution (2 mL, 0.9%), and placed for 60 min in a thermostat at 37°C. The final step was seeding of developed cultures on Lowenstein—Jensen and Finn-2 nutrient media [6]. The observation time was increased to three months because tubes with sealed stoppers were used.

Cytotoxic activity of essential oils was studied by survival of marine crustacean *A. salina*. The growth medium was marine brine (3.3%). Tested essential oil (20 mg) was dissolved in ethanol (2 mL), from which aliquots (500, 50, and 5 μL) were taken and diluted in brine (5 mL) to produce concentrations of 1000, 100, and 10 μg/mL. Each vial received 100 nymphs using a Pasteur pipette and was incubated at 25-27°C with artificial lighting, at the end of which the number of surviving nymphs was counted. The results were used to calculate the upper and lower toxicity limits of the half toxic dose for each sample.

REFERENCES

1. M. I. Goryaev, *Essential Oils of Flora of the USSR* [in Russian], Alma-Ata (1972).
2. R. B. Seidakhmetova, A. A. Beisenbaeva, G. A. Atazhanova, E. M. Suleimenov, R. N. Pak, A. T. Kulyyasov, and S. M. Adekenov, *Khim-farm. Zh.*, **3**, 27 (2002).

3. R. B. Seidakhmetova, R. N. Pak, A. A. Beisenbaeva, K. D. Rakhimov, and S. M. Adekenov, *Rastit. Resur.*, **1**, 102 (2002).
4. C. Bicchi, C. Frattini, and T. Sacco, *Phytochemistry*, **24**, 2440 (1985).
5. M. I. Goryaev, V. S. Bazalitskaya, and P. P. Polyakov, *Chemical Composition of Sages* [in Russian], Alma-Ata (1962).
6. V. A. Shkurupii, G. V. Mostovaya, N. V. Kazarinova, A. P. Ogirenko, S. D. Nikonov, A. V. Tkachev, and K. G. Tkachenko, *Probl. Tuberk.*, **4**, 36 (2002).