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# CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Acinos graveolens*

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The genus *Acinos* Miller (Lamiaceae) is represented by five species in the flora of Serbia. *Acinos graveolens* (syn. *Thymus graveolens* M. Bieb., *Calamintha graveolens* (M.B.) Benth, *Satureia graveolens* (M.B.) Stoj. et Stef.) is an annual species that was recently recorded in southern Serbia. In taxonomy *A. graveolens* is related to the West Mediterranean and North African taxa *A. rotundifolius* Pers., but in spite of this similarity the first one is treated as a separate species [1, 2].

Some species of genus *Acinos* Miller are employed in folk medicine as antiseptic, stimulant, tonic, and antispasmodic due to their beneficial effects on melancholy, coughs, toothache, sciatica, neuralgia, and gastrointestinal disorders [3–5].

The chemical composition of the oils of *A. hungaricus* [6, 7], *A. arvensis* [8–10], *A. suaveolens* [8, 9], *A. alpinus* [11, 12], *A. majoranifolius* [13], *A. rotundifolius* [9], and *A. troodi* [14] were previously studied. Concerning fatty acids, only the composition and content of fatty acids of *A. alpinus* and *A. hungaricus* have been reported in the literature [15]. Also, there is a paper which is related to the oil content, the qualitative and quantitative composition of tannins, and the qualitative analysis of the flavonoids [16]. As far as our literature survey could ascertain, the chemical composition and antimicrobial activity of the essential oil of *A. graveolens* have not been published previously.

The present work reports the composition and antimicrobial activity of the essential oil of *A. graveolens* (M. Bieb.) Link. (Lamiaceae) from Serbia for the first time.

The oil content of the aerial dried parts of *A. graveolens* was 0.06 mL (0.06%, v/w). Compared with other *Acinos* species whose oil yields were between 0.01–2.3%, it seems that *A. graveolens* belongs to a species with a rather low oil yield.

The results obtained after GC and GC-MS analysis of the oil are given in Table 1. Forty-five constituents were detected and identified in the oil. The main components were germacrene D (58.65%) and bicyclogermacrene (7.14%), while other compounds were present in less than 5%. The oil was characterized by a high percentage of sesquiterpene hydrocarbons (74.83%), followed by oxygenated sesquiterpenes (15.28%) and oxygenated monoterpenes (6.12%).

The results of the bioassay are presented in Table 2. The activity of *A. graveolens* diluted essential oil (in 1:10 dilution, w/v (mg/μL)) was a selective one. A significant reduction (with the fungicidal zone measuring 20 mm), in *C. albicans* growth was observed. The measured activity was comparable with the conventionally used the antifungal nystatin. Perhaps the anticandidal activity of the tested oil could be attributed to the high content of hydrocarbon sesquiterpenes germacrene D (58.65%) and bicyclogermacrene (7.14%). The above-mentioned sesquiterpenes were the most dominant components identified in Brazilian medicinal plant *Mikania glomerata* oil that was shown to possess strong anti-*Candida* activity as well [17]. However, bio-guided fractionation should be conducted in order to identify potential anticandidal active components. The investigated sample antibacterial activity was a moderate one. The diluted oil sample inhibited the growth of Gram-negative bacteria, while the Gram-positive ones were highly unsusceptible (antimicrobial activity was not observed), even though it is known that Gram-positive bacteria are more susceptible to essential oils compared to Gram-negative ones [18].

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TABLE 1. Composition of the Essential Oil of *Acinos graveolens*

| Compound* | KIE | % | Compound* | KIE | % |
|---|---|---|---|---|---|
| 1,8-Cineole | 1031.7 | 0.38 | Germacrene D | 1486.9 | 58.65 |
| Acetophenone | 1069.9 | 3.15 | Bicyclogermacrene | 1501.0 | 7.14 |
| Linalool | 1099.9 | 1.02 | $\beta$-Bisabolene | 1507.3 | 0.21 |
| Isoborneol | 1162.5 | 0.93 | *cis*-$\alpha$-Bisabolene | 1510.0 | 0.42 |
| Borneol | 1167.0 | Tr. | $\delta$-Cadinene | 1525.0 | 1.24 |
| *iso*-Menthol | 1179.3 | 0.37 | $\alpha$-Cadinene | 1538.1 | 0.07 |
| $\alpha$-Terpineol | 1187.6 | 0.92 | 11-Norbourban-1-one | 1556.3 | 0.28 |
| Myrtenol | 1198.5 | 0.24 | Germacrene D-4-ol | 1575.1 | Tr. |
| Pulegone | 1235.2 | 1.93 | Spathulenol | 1581.2 | 2.77 |
| Piperitone | 1249.9 | 0.19 | Caryophyllene oxide | 1584.8 | 2.77 |
| *trans*-Geraniol | 1258.2 | 0.15 | Viridiflorol | 1592.1 | 0.31 |
| Dihydroedulan | 1287.4 | 0.23 | Humulene epoxide II | 1606.4 | 0.16 |
| $\delta$-Elemene | 1337.2 | 0.11 | 1,10-di-*epi*-Cubenol | 1617.8 | 1.24 |
| $\beta$-Bourbonene | 1385.0 | 0.47 | Benzophenone | 1628.6 | 0.89 |
| $\beta$-Elemene | 1393.4 | 2.78 | $\tau$-Muurolol | 1642.5 | 2.36 |
| Dihydro-$\alpha$-ionone | 1407.9 | 0.15 | $\alpha$-Cadinol | 1657.6 | 2.43 |
| $\beta$-Caryophyllene | 1416.4 | Tr. | 14-Hydroxy-9-*epi*-(*E*)-caryophyllene | 1673.6 | 0.55 |
| $\beta$-Ylangene | 1419.7 | 1.02 | Germacra-4(15),5,10(14)-trien-1-$\alpha$-ol | 1688.0 | 0.95 |
| $\beta$-Copaene | 1430.0 | 0.43 | Mintsulfide | 1736.8 | 0.12 |
| *cis*-$\beta$-Farnesene | 1435.6 | 1.03 | $\beta$-Costol | 1767.3 | 0.14 |
| Isogermacrene D | 1445.3 | 0.19 | 6,10,14-Trimethyl-2-pentadecanone | 1845.8 | 0.13 |
| Selina-4(15),6-diene | 1455.0 | 0.48 | Phytol | 2115.6 | 0.16 |
| *trans*-$\beta$-Farnesene | 1465.2 | 0.85 | | | |

*Compounds listed in order of elution from a HP-5 MS column.
KIE: Retention index, experimentally determined (calibrated AMDIS).
Tr.: trace amount (<0.1%).

**Plant Material**. The material used for analysis is taken from the natural population in the blooming stage. The aerial parts of the plant were collected in May 2007 in the gorge of the Pcinja river (South Serbia, attitude ~500 m). Voucher specimens are deposited in the Herbarium of the Faculty of Biology and Botanical Garden "Jevremovac," University of Belgrade (BEOU) under the accession number 16140.

Dried and pulverized aerial parts of the plant (100 g) were hydrodistilled for 2.5 h using a Clevenger-type apparatus [6].

**Identification Procedure**. GC/FID analysis of the oils was carried out on a Hewlett-Packard HP-5890 Series II GC apparatus, equipped with a split-splitless injector and automatic liquid sampler (ALS), attached to an HP-5 column (25 m × 0.32 mm, 0.52 μm film thickness) and fitted to a flame ionization detector (FID). Carrier gas flow rate ($H_2$) was 1 mL/min, split ratio 1:30, injector temperature 250°C, and detector temperature 300°C, while column temperature was linearly programmed from 40–260°C (at a rate of 4°/min). Solutions of essential oil samples in ethanol (~1%) were consecutively injected by ALS (1 μL, split mode). Area percent reports, obtained as a result of standard processing of chromatograms, were used as base for quantification purposes.

The same analytical conditions as those mentioned for GC/FID were employed for GC/MS analysis, along with an HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness), using Hewlett-Packard HP G1800C Series II GCD system. Instead of hydrogen, helium was used as carrier gas. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in the *m/z* range 40–450. Sample solutions in ethanol (~1%) were injected by ALS (200 nL, split mode).

The components of the oil were identified by comparison of their mass spectra with those from Wiley275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1), compared with those from available literature, and used as additional tool to confirm the MS findings [19].

TABLE 2. Antimicrobial Activity[a] of the *Acinos graveolens* Oil*

| Zone | Microorganism | | | | |
|---|---|---|---|---|---|
| | *Acinos graveolens* 1:10 dilution | Ampicillin | Tetracycline | Streptomycin + Penicillin | Nystatin |
| | | | *E. coli* | | |
| C[b] | 15 | – | 30 | 23 | Nt. |
| S[c] | – | 19 | – | – | |
| | | | *S. enteritidis* | | |
| C | 15 | – | 28 | 19 | Nt. |
| S | – | 18 | – | – | |
| | | | *P. aeruginosa* | | |
| C | 17 | – | 27 | 21 | Nt. |
| S | – | 17 | – | – | |
| | | | *K. pneumoniae* | | |
| C | 18 | – | 27 | 20 | Nt. |
| S | – | – | – | – | |
| | | | *S. aureus* | | |
| C | 14 | – | 29 | 20 | Nt |
| S | – | 19 | – | – | |
| | | | *A. niger* | | |
| C | – | – | 23 | 20 | 17 |
| S | 14 | – | – | 32 | – |
| | | | *S. cerevisiae* | | |
| C | – | – | – | – | 17 |
| S | 18 | – | – | – | – |
| | | | *C. albicans* | | |
| C | 20 | – | 24 | 18 | 18 |
| S | – | – | – | 32 | – |

[a]Antimicrobial activities represented as the inhibition zones, in mm, including the disk diameter, 13 mm. Values for static zones represent the extra millimeters around the cidal zone (or the sole disk if no cidal activity) in which the growth of microorganisms was inhibited but in which the microorganisms were not killed; [b]Bacteri- and fungicidal zones; [c]Bacteri- and fungistatic zones; – no activity observed, Nt: not tested.
**Sarcina lutea* and *Bacillus subtilis* no activity observed.

**Antimicrobial Activity**. The *in vitro* antimicrobial activity of the *A. graveolens* essential oil was tested against a panel of laboratory control strains of the American Type Culture Collections (ATCC) Maryland, USA: Gram-negative: *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enteritidis* (ATCC 13076), and *Escherichia coli* (ATCC 25922); Gram-positive: *Staphylococcus aureus* (ATCC 6538), *Sarcina lutea* (ATCC 9341), and *Bacillus subtilis* (ATCC 6633); fungal organisms *Aspergillus niger* (ATCC 16404), *Saccharomyces cerevisiae* (ATCC 9763), and *Candida albicans* (ATCC 10231).

The disk diffusion method was employed for the determination of the antimicrobial activity of the essential oil according to the NCCLS recommendations [20]. Standard disks of ampicillin, tetracycline, streptomycin + penicillin, and nystatin (origin – Institute of Immunology and Virology "Torlak," 30 μg of the active component) were individually used as positive controls, while the disks were imbued with 50 μL of ether as a negative control (all tested microorganisms were completely nonsusceptible to control disks imbued with ether).

The diameters of the inhibition zones were measured in millimeters using a "Fisher-Lilly Antibiotic Zone Reader" (Fisher Scientific Co. USA). Each test was preformed in triplicate.

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