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# Composition and antimicrobial activity of the essential oil from *Galatella linosyris* (L.) Rchb. f. (Asteraceae)

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*Abstract*: An investigation of the chemical composition and antimicrobial activity of the essential oil of *Galatella linosyris* is presented. The chemical analysis (GC/MS, NMR) showed that sabinene (40 %), *β*-pinene (35.5 %), *α*-pinene (4.5 %), limonene (4 %), *γ*-muurolene (4 %), and (*E*)-caryophyllene (3.3 %) were dominant components in this oil. Microdilution assays were used to evaluate the minimum inhibitory concentration (*MIC*) and the minimum bactericidal/fungicidal concentrations (*MBC*/*MFC*). *G. linosyris* essential oil exhibited better antibacterial activity against some of the tested bacteria than antifungal activity.

*Keywords*: *Galatella linosyris*; essential oil; antimicrobial activity; GC/MS; NMR.

## INTRODUCTION

*Galatella linosyris* (L.) Rchb. f. (Bas. *Chrysocoma linosyris* L.; Syn. *Aster linosyris* (L.) Bernh.; *Linosyris vulgaris* Cass. Ex DC; *Crinitaria linosyris* (L.) Less.; *Crinitina linosyris* (L.) Soják), is a member of the Asteraceae (Compositae) family, distributed from the middle, southern and eastern part of Europe to southern Scandinavia in the North, extending locally northwards to England, and also in Central Russia.[1,2] This species, known as goldilocks aster, is a perennial herb with a decumbent to erect stem, 10–70 cm high. The leaves are lanceolate, sessile, and often glandular-punctate above. The capitula are small and grouped

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in dense corymbs. It grows on sunny rocks and stony slopes, on dry grasslands and cliffs, on calcareous soil.

*G. linosyris* has hitherto not been chemically investigated. From the plants of the *Galatella* genus, only the essential oil from *G. biflora* has been analyzed. The major components were *α*-pinene (34.7 %), (*E*)-*β*-ocimene (15.0 %), *β*-pinene (9.9 %), myrcene (7.4 %) and limonene (6.2 %).[3]

Based on the search for new alternatives for organic syntheses and natural biocontrol of bacteria and fungi, the objective of this study was to investigate the antibacterial and antifungal activity of the essential oil from *G. linosyris* against food poisoning and plant, animal and human pathogenic bacteria and fungi.

## EXPERIMENTAL

### *Plant material*

The plant material was collected in Deliblatska peščara, Serbia, during September 2010. A voucher specimen (BEOU16403) is deposited at the Herbarium of the Botanical Garden "Jevremovac," Faculty of Biology, University of Belgrade, Serbia.

### *Isolation of the essential oil*

The air-dried plants (300 g) of *G. linosyris* were submitted for 3 h to water-distillation using a Clevenger apparatus. The obtained essential oil (1.2 mL) was stored at +4 °C until tested and analyzed. The qualitative and quantitative analyses of the oils were performed using nuclear magnetic resonance (NMR) spectroscopy, gas chromatography (GC) and GC coupled to mass spectroscopy (GC/MS).

### *GC and GC/MS*

The GC and GC/MS analyses were performed on an Agilent 7890A GC equipped with a 5975C inert XL EI/CI mass selective detector (MSD) and a flame ionization detector (FID) connected by a capillary flow technology 2-way splitter with make-up. An HP-5MSI capillary column (30m×0.25mm×0.25μm) was used. The temperature of the GC oven was programmed from 60 °C to 300 °C at 3 °C $min^{-1}$ and held for 10 min. Helium was used as the carrier gas at 16.255 psi (constant pressure mode). The sample was analyzed in the splitless mode. The injection volume was 1 μL. The FID temperature was 300 °C. The MS data was acquired in the EI mode, with a scan range 30–550 *m*/*z*; the source temperature was 230 °C and the quadrupole temperature was 150 °C. The solvent delay was 3 min.

### *NMR*

The NMR spectra were acquired on a Bruker Avance DRX 500 MHz instrument with a 5 mm inverse detection probe, in $CDCl_3$ as the solvent, at 298 K. The spectra were referenced to tetramethylsilane (TMS), chemical shifts are given in $\delta$ (ppm), and coupling constants are reported in Hz. Two-dimensional experiments (COSY, HSQC, and HMBC) were recorded with standard Bruker pulse sequences.

### *Antibacterial activity*

The following Gram-negative bacteria: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Listeria monocytogenes* (NCTC 7973), *Enterobacter cloacae* (human isolate) and the following Gram-positive bacteria: *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240) and *Staphylococcus aureus* (ATCC 6538) were used.

The bacterial cell suspension was adjusted with sterile saline to a concentration of approximately $1.0\times10^5$ CFU (colony forming units) in a final volume of 100 μL per well. The microplates were incubated for 24 and 48 h at 37 °C. The lowest concentrations without visible growth (under a binocular microscope) were defined as the minimum inhibition concentration (*MIC*) values. The minimum bactericidal concentration (*MBC*) values were determined by serial subcultivation of 2 μL in microtitre plates containing 100 μL of broth per well and further incubation for 24 and 48 h at 37 °C. The lowest concentration with no visible growth was defined as the *MBC*, respectively indicating 99.5 % killing of the original inoculum. Each experiment was repeated in triplicate. Streptomycin was used in the positive controls (1 mg $mL^{-1}$).

*Antifungal activity*

For the antifungal bioassays, the following fungi were used: *Aspergillus fumigatus* (plant isolate), *A. niger* (ATCC 6275), *A. versicolor* (ATCC 11730), *A. ochraceus* (ATCC 12066), *Penicillium funiculosum* (ATCC 36839), *P. ochrochloron* (ATCC 9112) and *Trichoderma viride* (IAM 5061).

The fungal spores were washed from the surface of agar plates with sterile 0.85 % saline containing 0.1 % Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a spore concentration of approximately $1.0 \times 10^5$ in a final volume of 100 μL per well. *MIC* determinations were performed by a serial dilution technique using 96-well microtiter plates. The essential oil was dissolved in 5 % DMSO solution containing 0.1 % Tween 80 (v/v) (1 mg $mL^{-1}$) and added into the broth medium with inoculum. The microplates were incubated for 72 h at 28 °C. The lowest concentrations without visible growth (at the binocular microscope level) were defined as *MIC* values. The minimum fungicidal concentration (*MFC*) values were determined by serial subcultivation of 2 μL into microtiter plates containing 100 μL of broth per well and further incubation for 72 h at 28 °C. The lowest concentration with no visible growth was defined as the *MFC* indicating 99.5 % killing of the original inoculum. The commercial fungicide ketoconazole was used as positive controls (0.60–25 μg $mL^{-1}$).

The optical density of each well was measured at a wavelength of 655 nm using a Microplate manager 4.0 (Bio-Rad Laboratories) and compared with the corresponding blank and positive control. An aqueous solution of 3 % INT color *p*-iodonitrotetrazolium violet (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride; Sigma) was added to each well and stored for another 3 h for incubation at 37 °C (for bacteria) and 28 °C (for fungi). Changes in color from yellow to a pinkish were indicative that there was no antimicrobial activity of tested oils, whereas no changes in color after 3 h indicated antimicrobial activity of the tested oil. Each experiment was repeated in triplicate.

## RESULTS AND DISCUSSION

The components of the oil were identified by comparison of their mass spectra to those from Adams, Wiley 7, and NIST05 libraries. The identification was confirmed by the retention time lock (RTL) method and the RTL Adams database. For quantification purpose, area percent data registered by the FID were used. GC and GC/MS analyses showed a total of 18 compounds in the essential oil of *G. linosyris* (Table I). The dominant compounds were sabinene (40 %) and *β*-pinene (35.5 %), followed by *α*-pinene (4.5 %), limonene (4 %), *γ*-muurolene (4 %) and (*E*)-caryophyllene (3.3 %). The chemical composition of the essential

oil from *G. linosyris* was similar to that of *G. biflora*,[3] only differences in the component distribution were registered.

TABLE I. Chemical composition of *G. linosyris* essential oil (*MIC* and *MBC* in μg mL$^{-1}$)

| Compound | Retention time, min | *RI* | % |
|---|---|---|---|
| *α*-Thujene | 5.622 | 910 | 0.24 |
| *α*-Pinene | 5.807 | 918 | 4.46 |
| Sabinene | 6.898 | 968 | 39.98 |
| *β*-Pinene | 7.011 | 973 | 35.46 |
| Myrcene | 7.400 | 990 | 0.77 |
| Terpinene | 8.265 | 1016 | 0.75 |
| Limonene | 8.676 | 1027 | 4.02 |
| *Z*-Ocimene | 8.994 | 1034 | 0.14 |
| *E*-Ocimene | 9.366 | 1044 | 0.64 |
| *γ*-Terpinene | 9.768 | 1054 | 1.24 |
| Terpinolene | 10.923 | 1086 | 0.20 |
| Terpinylacetate | 22.263 | 1351 | 0.14 |
| *β*-Elemene | 24.124 | 1393 | 0.97 |
| *E*-Caryophyllene | 25.298 | 1420 | 3.25 |
| *α*-Humulene | 26.764 | 1459 | 0.18 |
| *γ*-Muurolene | 27.943 | 1483 | 3.98 |
| *γ*-Patchoulene | 28.603 | 1499 | 0.43 |
| Premnaspirodiene | 28.957 | 1508 | 0.63 |
| Total | | | 97.49 |

The identities of sabinene and *α*- and *β*-pinene were confirmed by 2D NMR analysis of the whole essential oil. Assignment of the $^1$H- and $^{13}$C-NMR signals was achieved by characteristic COSY, HSQC, and HMBC correlations (Figs. 1––3). The 2D techniques also enabled the resolution of the overlapped $^1$H- and $^{13}$C-NMR signals of each detected compound. The obtained chemical shifts were comparable to those from the literature.[4,5]

*Sabinene*. $^1$H-NMR (500 MHz, $CDCl_3$, *δ* / ppm): 0.64 (2H, *d*, *J* = 5.2 Hz), 0.88 (3H, *d*, *J* = 6.8 Hz), 0.94 (3H, *d*, *J* = 6.8 Hz), 1.48 (1H, *sept*, *J* = 6.8 Hz), 1.58 (1H, *t*, *J* = 5.2 Hz), 1.70 (2H, *m*), 2.01 (1H, *m*), 2.14 (1H, *m*), 4.61 (1H, *m*), 4.80 (1H, *m*). $^{13}$C-NMR (125 MHz, $CDCl_3$, *δ* / ppm): 16.0, 19.6, 19.7, 27.4, 28.9, 30.1, 32.6, 37.5, 101.5, 154.4.

β-*Pinene*. $^1$H-NMR (500 MHz, $CDCl_3$, *δ* / ppm): 0.72 (3H, *s*), 1.23 (3H, *s*), 1.42 (1H, *d*, *J* = 9.8 Hz), 1.82 (2H, *m*), 1.97 (1H, *m*), 2.24 (1H, *m*), 2.31 (1H, *m*), 2.45 (1H, *t*, *J* = 5.4 Hz), 2.53 (1H, *m*), 4.55 (1H, *m*), 4.62 (1H, *m*). $^{13}$C-NMR (125 MHz, $CDCl_3$, *δ* / ppm): 21.8, 23.5, 23.5, 26.1, 26.9, 40.4, 51.7, 105.9, 152.2.

α-*Pinene*. $^1$H-NMR (500 MHz, $CDCl_3$, *δ* / ppm): 0.83 (3H, *s*), 1.26 (3H, *s*), 1.16 (1H, *d*, *J* = 8.5 Hz), 1.65 (3H, *m*), 1.93 (1H, *m*), 2.07 (1H, *m*), 2.17 (1H, *m*), 2.21 (1H, *m*), 2.33 (1H, *m*), 5.18 (1H, *t* of *sex*, *J* = 3.0; 1.5 Hz). $^{13}$C-NMR (125 MHz, $CDCl_3$, *δ* / ppm): 20.8, 23.0, 26.3, 31.3, 31.3, 38.1, 40.5, 47.0, 115.9, 144.4.

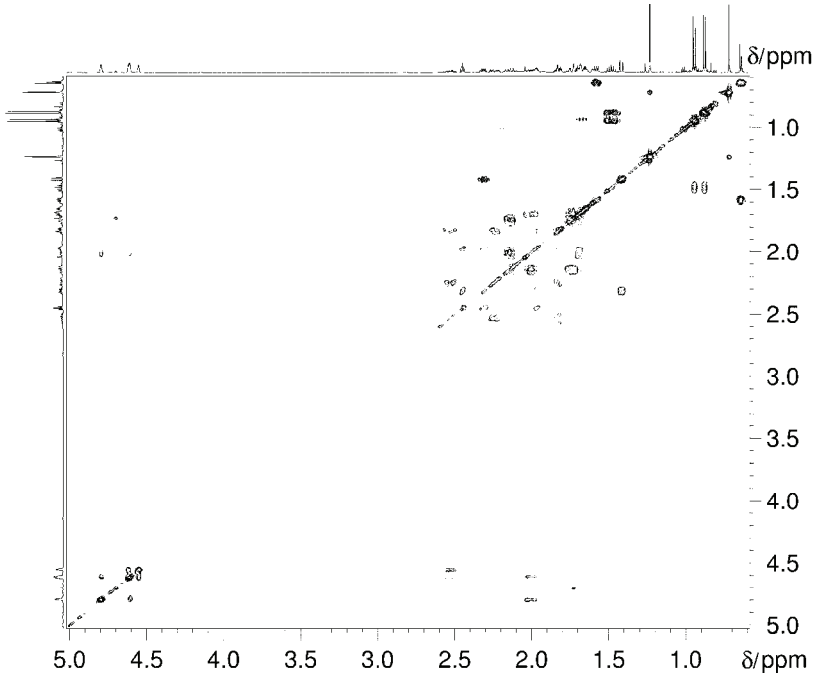


Fig. 1. COSY spectrum of *G. linosyris* essential oil.

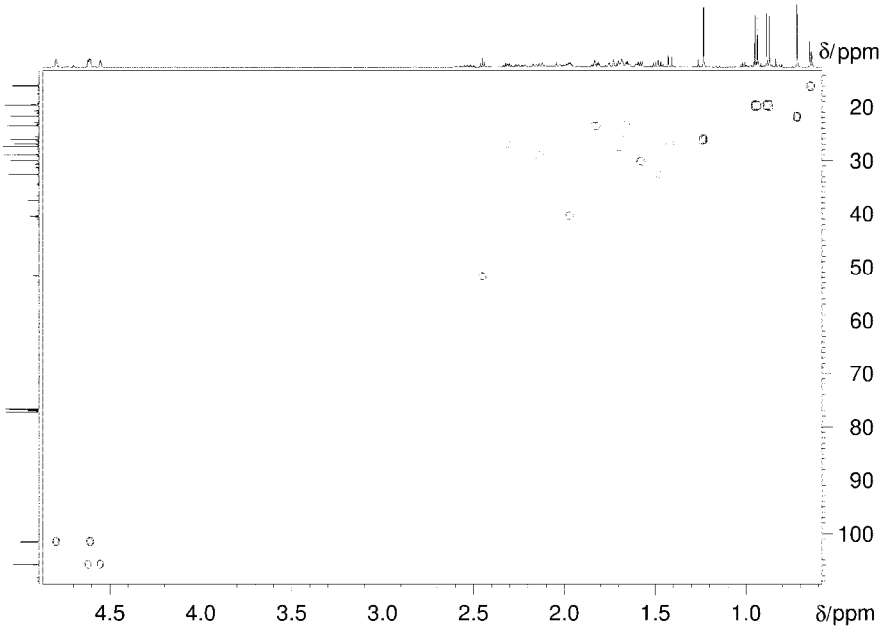


Fig. 2. HSQC spectrum of *G. linosyris* essential oil.

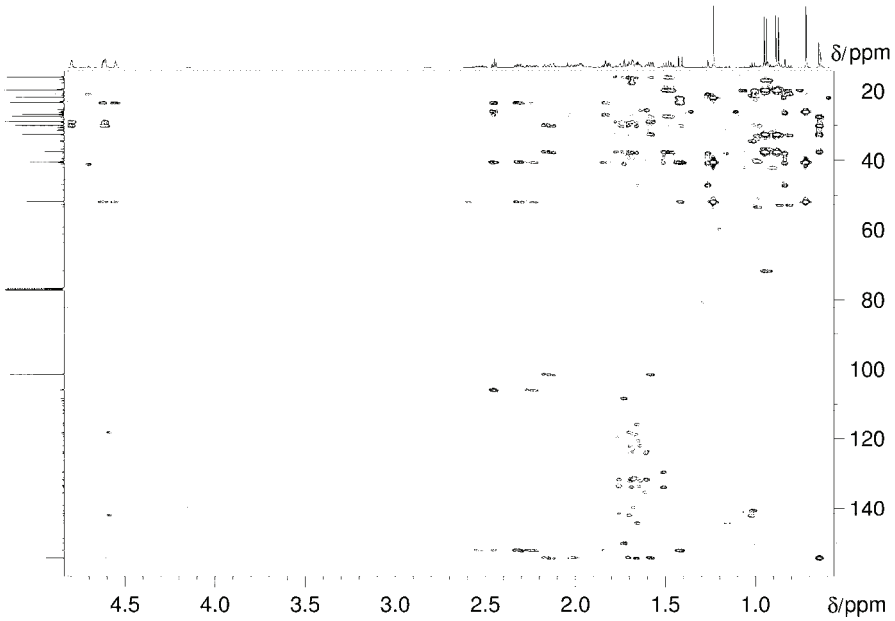


Fig. 3. HMBC spectrum of *G. linosyris* essential oil.

The results of the determination of the antibacterial and antifungal activity of the tested essential oil are given in Tables II and III, respectively. The bacteria were proved to be more sensitive than fungi to the effect of the essential oil.

TABLE II. Antibacterial activity of *G. linosyris* essential oil and streptomycin (*MIC* and *MBC* in μg $mL^{-1}$)

| Bacterium | Concentration type | *G. linosyris* | Streptomycin[a] |
|---|---|---|---|
| *S. aureus* | *MIC* | – | 3.13 |
| | *MBC* | – | 6.25 |
| *B. cereus* | *MIC* | 12.50 | 1.25 |
| | *MBC* | 25.00 | 2.50 |
| *M. flavus* | *MIC* | – | 0.63 |
| | *MBC* | – | 1.25 |
| *L. monocytogenes* | *MIC* | – | 12.50 |
| | *MBC* | – | 25.00 |
| *P. aeruginosa* | *MIC* | 6.00 | 1.60 |
| | *MBC* | 12.50 | 3.13 |
| *E. cloacae* | *MIC* | – | 0.63 |
| | *MBC* | – | 1.25 |
| *S. typhimurium* | *MIC* | 25.00 | 1.25 |
| | *MBC* | 50.00 | 2.50 |
| *E. coli* | *MIC* | 6.00 | 0.63 |
| | *MBC* | 25.00 | 1.25 |

[a]Stock solution 1 mg $mL^{-1}$

TABLE III. Antifungal activity of *G. linosyris* essential oil and ketoconazole (*MIC* and *MFC* in μg mL$^{-1}$)

| Fungus | Concnetration type | *G. linosyris* | Ketoconazole[a] |
|---|---|---|---|
| *A. versicolor* | *MIC* | 25.00 | 0.63 |
| | *MFC* | 100.00 | 5.00 |
| *A. ochraceus* | *MIC* | 50.00 | 5.00 |
| | *MFC* | 100.00 | 25.00 |
| *A. niger* | *MIC* | 100.00 | 25.00 |
| | *MFC* | 200.00 | 25.00 |
| *A. fumigatus* | *MIC* | 25.00 | 2.50 |
| | *MFC* | 100.00 | 5.00 |
| *P. ochrochloron* | *MIC* | 25.00 | 5.00 |
| | *MFC* | 100.00 | 10.00 |
| *P. funiculosum* | *MIC* | 25.00 | 0.63 |
| | *MFC* | 150.00 | 1.25 |
| *T. viride* | *MIC* | 25.00 | 25.00 |
| | *MFC* | 200.00 | – |

[a]Stock solution 1 mg mL$^{-1}$

The oil exhibited *MIC* values against the tested bacteria in the concentration range 6.0–25.0 μg mL$^{-1}$, and *MBC* values in range of 12.5–50.0 μg mL$^{-1}$. The most sensitive bacteria was *P. aeruginosa* with an *MIC* of 6 μg mL$^{-1}$ and an *MBC* of 12.5 μg mL$^{-1}$, while *S. typhimurium* was only slightly sensitive with an *MIC* of 25.0 μg mL$^{-1}$ and an *MBC* of 50.0 μg mL$^{-1}$. The bacteria *S. aureus*, *M. flavus*, *L. monocytogenes* and *E. coli* were resistant to this oil. Streptomycin showed activity against the tested bacteria in the concentration range 0.63–25.0 μg mL$^{-1}$.

The essential oil from *G. linosyris* possessed antifungal activity with *MIC* values in the range 25.0–100.0 μg mL$^{-1}$ and *MFC* values in the range 100.0––200.0 μg mL$^{-1}$ (Table III). The commercial preparation of the fungicidal agent, ketoconazole showed *MIC* values in the range 0.63–25.0 μg mL$^{-1}$ and *MFC* values in the range of 1.25–25.0 μg mL$^{-1}$ (Table III).

From the chemical analysis of this oil, it can be seen that hydrocarbons were the dominant components (approx. 80 %). Hydrocarbons tend to be relatively inactive regardless of their structural type, and this inactivity is closely related to their limited hydrogen bonding capacity and water solubility. This suggested that the components present in great proportions were responsible for a large share of the total activity.

From the results given above, it can be concluded that the studied essential oil expressed antimicrobial activity. The antifungal activity was slightly lower in comparison to the antibacterial effect against certain bacteria. The oil exhibited lower antimicrobial activity than did the used commercial antimicrobial agents. The growth of tested microorganism responded differently to the essential oil,

which indicates that different components may have different modes of action or that the metabolism of some microorganisms is better able to overcome the effect of the oil or adapt to it.

The obtained results clearly demonstrate that the tested oil presents potential for medical procedures and for the food, cosmetics and pharmaceutical industries.

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ИЗВОД

## САСТАВ И АНТИМИКРОБНА АКТИВНОСТ ЕТАРСКОГ УЉА ИЗ *Galatella linosyris* (L.) Rchb. f. (ASTERACEAE)

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У овом раду је приказано испитивање хемијског састава и антимикробне активности етарског уља биљне врсте *Galatella linosyris*. Хемијском анализом (GC–MS и NMR) је утврђено да су главни састојци овог етарског уља сабинен (40 %), $\beta$-пинен (35,5 %) , $\alpha$-пинен (4,5 %), лимонен (4 %), $\gamma$-мууролен (4 %) и (*E*)-кариофилен (3,3 %). За процену минималне инхибиторне концентрације (*MIC*) и минималне бактерицидне/фунгицидне концентрације (*MBC*/*MFC*) коришћени су тестови микроразблажења. Етарско уље *G. linosyris* је показало бољу антибактеријску него антифунгалну активност.

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