# The identification and quantification of bioactive compounds from the aqueous extract of comfrey root by UHPLC–DAD–HESI–MS method and its microbial activity

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*Abstract*

The qualitative, quantitative and microbial determination of the aqueous extract of comfrey root was done in this paper. The qualitative and quantitative analyses were done by the UHPLC–DAD–HESI–MS method. Allantoin, rosmarinic acid and ellagic acid were identified as major bioactive compounds and their quantification was also done. The obtained results showed a high content of allantoin, ellagic acid and rosmarinic acid (8.91, 7.4 and 12.8%, respectively) which indicated that the comfrey root can be used as a source for the isolation of these three compounds. The results obtained by the determination of the antimicrobial activity showed that *Escherichia coli* ATCC 8739 and *Salmonela typhimirium* ATCC 6538 were most sensitive to the aqueous extract of comfrey root. The results showed that allantoin did not express the antimicrobial activity on all the investigated bacteria species, and based on this it can be concluded that allantoin is not responsible for the antimicrobial activity of the aqueous extract of comfrey root.

*Keywords*: comfrey root, *Symphytum officinale* L., UHPLC–DAD–HESI–MS, microbial activity.

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Comfrey (*Symphytum officinale* L.) is a perennial herb which belongs to the genus *Symphytum* of the *Boraginaceae* family. It is a weed plant that is usually found in some parts of Asia, Europe and in North America [1,2]. Previous studies have shown that comfrey possesses good analgesic, anti-inflammatory, astringent, expectorant, antifungal and decongestant properties [3–5]. In folk medicine, the comfrey root has been used externally as a traditional medicinal plant (as an ointment, compress, or alcoholic fermentation) for treating fractures, strains, thrombophlebitis and haematoma, and internally (as tea, tincture or infusions) in treating gastro-intestinal and respiratory tract diseases [6]. On the other hand, the leaves and stems have also been used for treating the same disorders, as well as for treating rheumatism and gout [7].

Previous studies showed that these beneficial properties of comfrey are the result of the presence of numerous bioactive compounds [3,8]. It is known that comfrey contains allantoin, 18 amino acids, A, B and C vitamins, ellagic acid, auxin, triterpenoids, tannins, rosmarinic acid, steroidal saponins, inulin, pyrrolizidine alkaloids, calcium, potassium, iron, sulfur, copper, selenium. Hydrocolloid polysaccharides, which represent immunomodulatory substances, were also detected in comfrey [9,10].

Although comfrey consists of numerous different compounds, allantoin, ellagic acid and rosmarinic acid are probably the most important for its beneficial activities [11]. Allantoin stimulates the metabolic process in the subcutaneous tissue and stimulates the cell growth (proliferation), which results in epithelialization and a protective effect on the skin. It also strongly promotes the cell growth in bone cells (the bone fracture) and connective tissues (tendons, ligaments). Allantoin has a moisturizing and keratolytic effect, increases the water content of the extracellular matrix and improves desquamation of upper layers of the dead skin cells, increases the smoothness of the skin, promotes the cell proliferation and wound healing. It exhibits a mitigating, anti-irritating and protective effect on the skin by forming a complex with irritants and sensitizing agents [12]. On the other hand, rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, and it is consistent in *Boraginaceae* and *Lamiaceae* plant species. Rosmarinic acid possesses antioxidant activities [13,14], antiviral, anti-inflammatory effects and shows a very low toxicity [15] and antitumor potential. It is widely used for the preparation of many products in pharmaceutical, cosmetic and food

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industry. The rosemary essential oil has long been known for its antimicrobial and antivirus activities [16]. As well as the two previously mentioned compounds, ellagic acid also possesses many beneficial activities. It may occur in a free form in many fruits and vegetables and it has been investigated by researchers for a series of pharmacological properties such as antiviral, antioxidant, antimutagenic and anticancer activities [17,18]. In addition, ellagic acid has the effect to reduce a heart disease, birth defects, liver problems, the side effects of chemotherapy in men with advanced prostate cancer and it has also shown to be an effective inhibitor of *in vitro* lipid peroxidation [19,20].

Taking into account the multiple significance of comfrey, one of the aims of this study was to develop a new method, UHPLC–DAD–HESI–MS, for the qualitative analysis of the aqueous extracts of comfrey root and quantitative determination of allantoin, ellagic acid and rosmarinic acid as major bioactive compounds which are important pharmacodynamic properties of comfrey extract. Comfrey, as a source of bioactive compounds with potentially beneficial biological effects, is poorly investigated, but the results about its antimicrobial activity were not found in literature data [11]. Therefore, in this study, the microbial activity of the aqueous extracts of comfrey root was investigated and it was compared to the standard of allantoin that is the main pharmacological component in comfrey root.

## MATERIALS AND METHODS

### Reagents

Acetonitrile and water were purchased from Fisher Chemical (LC-MS and HPLC grade, respectively). Allantoin, ellagic acid and rosmarinic acid standards were purchased from Sigma-Aldrich. Formic acid was purchased from Carlo Erba (Italy).

### The plant material

In this study, we used an air-dried comfrey root (*Symphyti* radix) which was obtained from the Institute for Medicinal Plant Research "Dr Josif Pančić". Dried root was milled in a laboratory disintegrator (the laboratory electric mill "Braun Aromatic KSM2").

### Extract preparation

Chopped and homogenized plant material (30 g) was packed in the filter paper cocoon and placed in the Soxhlet extraction apparatus. 300 mL of water was measured in a reception vessel and the extraction was carried out at the boiling temperature of the solvent for 240 min. The solvent was removed by evaporation on a rotary vacuum evaporator at 50 °C. The resulting extract was dried in a laboratory oven at 50 °C to constant weight.

### UHPLC–DAD–HESI–MS analysis

All the experiments were performed by using a Thermo Scientific liquid chromatography system (UHPLC) composed of a quaternary pump with a degasser, a thermostated column compartment, an autosampler, and a diode array detector connected to LCQ fleet ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with heated electrospray ionisation (HESI). Xcalibur (version 2.2 SP1.48) and LCQ Fleet (version 2.7.0.1103 SP1) software were used for the instrument control, data acquisition and data analysis. Separations were performed on a Hypersil gold C18 (50 mm×2.1 mm, 1.9 μm) from Thermo Fisher Scientific.

UHPLC–DAD–HESI–MS analysis was performed according to Kečkeš *et al*. (2013) with slight modifications [21]. The mobile phase consisted of (A) water + 0.1% formic acid and (B) acetonitrile + 0.1% formic acid. A linear gradient program at the flow rate of 0.350 mL/min was used for 0–2 min from 10 to 20% (B), 2–4.5 min from 20 to 90% (B), 4.5–4.8 min 90% (B), and 4.8––4.9 min from 90 to 10% (B), 4.9–12.0 min 10% (B). The injection volume was 10 μL, the column temperature was maintained at 25 °C, the separated compounds were detected at the wavelength of 272, 294, 308 and 360 nm, and each online spectrum was recorded within the range of 200–800 nm. The mass spectrometer was operated in a positive mode. HESI-source parameters were as follows: the source voltage 5.0 kV, the capillary voltage 46.00 V, the tube lens voltage 85.00 V, the capillary temperature 400 °C, the sheath and the auxiliary gas flow ($N_2$) 32 and 12 (arbitrary units). MS spectra were acquired by full range acquisition covering 150–700 *m*/*z*. For the fragmentation study, a data dependant scan was performed by deploying the collision-induced dissociation (CID). The normalized collision energy of the collision-induced dissociation (CID) cell was set at 25 eV.

### Cultures of microorganisms used for the determination of microbial activity

To evaluate the *in vitro* microbial activity of the aqueous extract of comfrey root and allantoin standards, laboratory control microorganism strains that belong to the American Type Culture Collection (ATCC, Rockville, MD, USA) were used. *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) were used as Gram-positive bacteria, *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27857) and *Salmonella typhimurium* (ATCC 14028) were used as Gram-negative bacteria, and *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 10231) were used as fungi.

The cultures of microorganisms were stored in the microbiology laboratory of the Faculty of Science in Niš

at –20 °C under appropriate conditions, subcultured twice immediately before the use in the microdilution test, when fresh suspensions of microorganisms were prepared. Standardized suspensions were prepared by flushing microorganisms with 0.9% NaCl solution with oblique and agar dilution to the appropriate number of cells, also saline. The number of microorganisms was determined with the turbitimer.

**Determination of microbial activity**

To determine the minimum inhibitory concentration (*MIC*) of the aqueous extract of comfrey root and allantoin, the microdilution method was applied and it was performed in microtiter plates with 96 conical holes [22,23]. The suspension was inoculated with bacteria that were prepared from the cultures of the appropriate microorganisms which were one day old, and the number of colonies in the suspension culture adjusted to 0.5 McFarland's turbitimer standards (bacterial colonies of microorganisms $2.0\times10^{-6}$ CFU $mL^{-1}$ and fungal spores $2.0\times10^{5}$). The initial concentration of the comfrey extract and allantoin were 40 and 10 mg/mL, respectively. After double dilution series *in situ*, the concentration of the samples was in the range of $2.2\times10^{-6}$ to 10 mg/mL for the aqueous solution of allantoin and $1.96\times10^{-2}$ to 40 mg/mL for the aqueous extract of comfrey root. Two columns in each plate were used as controls. One column was positive, and it included the antibiotic with a wide range of activities (doxycycline, a series of dilutions of 50–0.02 mg/mL). The second column contained the solvent. The growth of microorganisms was followed by the addition of 10 mL resasurine (BHD Laboratory Supplies) as an indicator (prepared by dissolving the tablets of 270 mg in 40 mL of sterile distilled water) into each indentation in the inoculated microtiter plate filled with the samples which were to be tested. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. After the incubation, the *MIC* values were determined based on the color change indicator. The color change from purple to pink or total bleaching was considered as a positive result. The lowest concentration where there was a change of color (bleaching) was adopted as the *MIC* value.

The minimum bactericidal/fungicidal concentration (*MBC*/*MFC*) was determined by streaking the contents of each microtiter plate recess in which the described change of the indicator color in the sterile surface of the nutrient agar prepared in Petri dishes was observed. Petri dishes were incubated at 37 °C for 24 h (bacteria) and at 28 °C for 48 h (fungi). After the incubation, the minimum bactericidal – fungicidal concentration was evaluated as one, where there was a lack of growth of 99.9% of the initial cell inoculum. All the tests were done in triplicate.

## RESULTS AND DISCUSSION

### UHPLC–DAD–HESI–MS analysis of the aqueous extract of comfrey root

Previous studies have shown that comfrey possesses numerous beneficial properties due to the presence of many compounds with biological activity. But unfortunately, the studies have also shown that this herb is also potentially toxic due to the presence of toxins in comfrey which include pyrrolizidine alkaloids (PAs) such as lycopsamine, echimidine and lasiocarpine, and their *N*-oxides [24,25]. Based on this, it can be concluded that the determination of the composition of the aqueous extract of comfrey root is of great importance. For the identification of the composition of the aqueous extract of comfrey root, a new UHPLC–DAD––HESI–MS method was developed and applied. All compounds were identified based on retention times, UV––Vis absorption spectrum and mass spectra by matching their molecular ions obtained by LC–HESI–MS and LC–MS/MS methods with theoretical molecular weights from literature data [6,21,25–27].

Figure 1 shows the UHPLC–DAD chromatogram of the aqueous extract of comfrey root at 272 nm. In Table 1, the identified compounds from the extract are shown, labeled as peaks 1–12 following the elution orders in the UHPLC–DAD chromatograms (mass spectra are not shown). In addition, structural formulas of the identified compounds in the extract are shown in Figure 2.

The first peak at retention time of 0.56 min was identified as allantoin. The presence of pseudomolecular ion $[M+H]^+$ in the positive ion mode at 159 *m*/*z* and its ion fragment at 116 *m*/*z* indicates that the first peak is allantoin. The presence of allantoin in the extract was also confirmed by standard reference.

Rosmarinic acid was identified as peak 2 at retention time of 5.31 min. MS Fragmentation of pseudomolecular ion $[M–H]^-$ in the negative mode at 359 *m*/*z* showed two ion fragments at 161 and 133 *m*/*z*. The presence of rosmarinic acid was also confirmed by standard reference. Peak 3 at retention time of 5.95 min was identified as lycopsamine. Lycopsamine contains the fragment ion at 120 *m*/*z*, which is a characteristic of the retronecine moiety, and represents the basic structure from which it is derived [25]. MS Fragmentation of lycopsamine showed two characteristic fragmenation ions at 120 and 138 *m*/*z*, which represent characteristic fragmentation ions of retronecine-type alkaloids. 5-*O*-Feruloylquinic acid was identified as peak 4 at retention time of 6.27 min. The presence of pseudomolecular ion $[M+H]^+$ in the positive mode at 369 *m*/*z* and ion fragments at 193 and 173 *m*/*z* after MS fragmentation indicates that 5-*O*-feruloylquinic acid is present in the aqueous extract of comfrey root. Peak 5

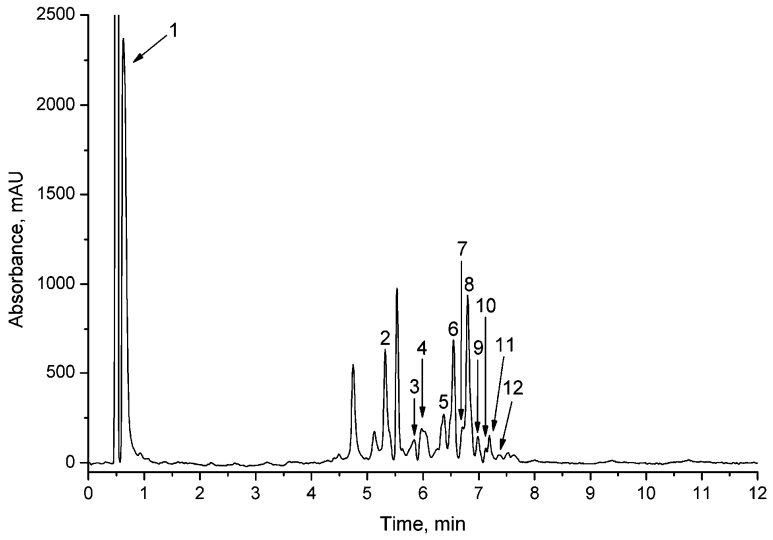


*Figure 1. UHPLC Chromatogram of the aqueous extract of comfrey root (Symphytum officinale L.) detected at 272 nm.*

*Table 1. The identified compounds in the aqueous extract of comfrey root*

| Peak No. | $t_R$ / min | Ionization mode | Pseudomolecular ion | MS/MS | Compound |
|---|---|---|---|---|---|
| 1 | 0.56 | + | 159 | 116 | Allantoin |
| 2 | 5.31 | – | 359 | 161, 133 | Rosmarinic acid |
| 3 | 5.95 | + | 300 | 120, 138 | Lycopsamine |
| 4 | 6.27 | + | 369 | 193, 173 | 5-*O*-Feruloylquinic acid |
| 5 | 6.48 | + | 273 | 255, 227, 153 | Pinobanksin |
| 6 | 6.62 | + | 291 | 245, 205, 179 | Epicatechin |
| 7 | 6.72 | + | 455 | 291, 163 | *p*-Coumaroyl-hexoside-methylglutarate |
| 8 | 6.79 | + | 539 | 455, 291, 163 | Derivate of *p*-coumaroyl-hexoside--methylglutarate |
| 9 | 6.97 | – | 315 | 151 | Quercetin-3-methylether |
| 10 | 7.10 | – | 301 | 257, 229 | Ellagic acid |
| 11 | 7.21 | + | 319 | 279, 149 | Myricetin |
| 12 | 7.51 | + | 412 | 394, 336, 238, 220, 120 | Lasiocarpine |

at retention time of 6.48 min with pseudomolecular ion $[M+H]^+$ at 273 *m*/*z* was identified as pinobanksin. After MS fragmentation, three ion fragments at 255, 227 and 153 *m*/*z* were present. Based on the obtained results from MS fragmentation it can be concluded that this compound is pinobanksin. Pseudomolecular ion $[M+H]^+$ in the positive mode at 291 *m*/*z* and its ion fragments at 245, 205 and 179 *m*/*z* indicated that peak 6 at retention time of 6.62 min was epicatechin. Peaks 7 and 8 were identified as *p*-coumaroyl-hexosidemethylglutarate and its derivate, respectively. After MS fragmentation, both compounds showed the same ion fragments at 291 and 163 *m*/*z* whereby peak 8 showed the additional ion fragment at 455 *m*/*z.*

Peak 9 at retention time of 6.97 min was identified as quercetin-*3*-methylether. Based on its pseudomolecular ion $[M–H]^–$ in the negative mode at 315 *m*/*z* and its ion fragment at 151 *m*/*z* it can be concluded that quercetin-*3*-methylether is present in the aqueous comfrey root extract. Ellagic acid was identified as peak **10** at retention time of 7.10 min. Ellagic acid and quercetin have a similar pseudomolecular ion $[M–H]^–$ in the negative mode at 301 *m*/*z*, but their MS fragmentation is different. After MS fragmentation, based on the presence of ion fragments at 257 and 229 *m*/*z* it can be concluded that peak 10 is ellagic acid. The presence of ellagic acid in the aqueous extract of comfrey root was confirmed by standard reference. Myricetin was identified as peak 11 at retention time of 7.21 min. In the positive mode its pseudomolecular ion $[M+H]^+$ at 319 *m*/*z*, and ion fragments at 279 and 149 *m*/*z* confirmed that myricetin was present in the aqueous extract of comfrey root. In the analysis of the aqueous extract of comfrey root, lasiocarpine (peak 12) was also detected. The presence of pseudomolecular ion $[M+H]^+$ in the positive mode at 412 *m*/*z*, and ion fragment at 120 *m*/*z*

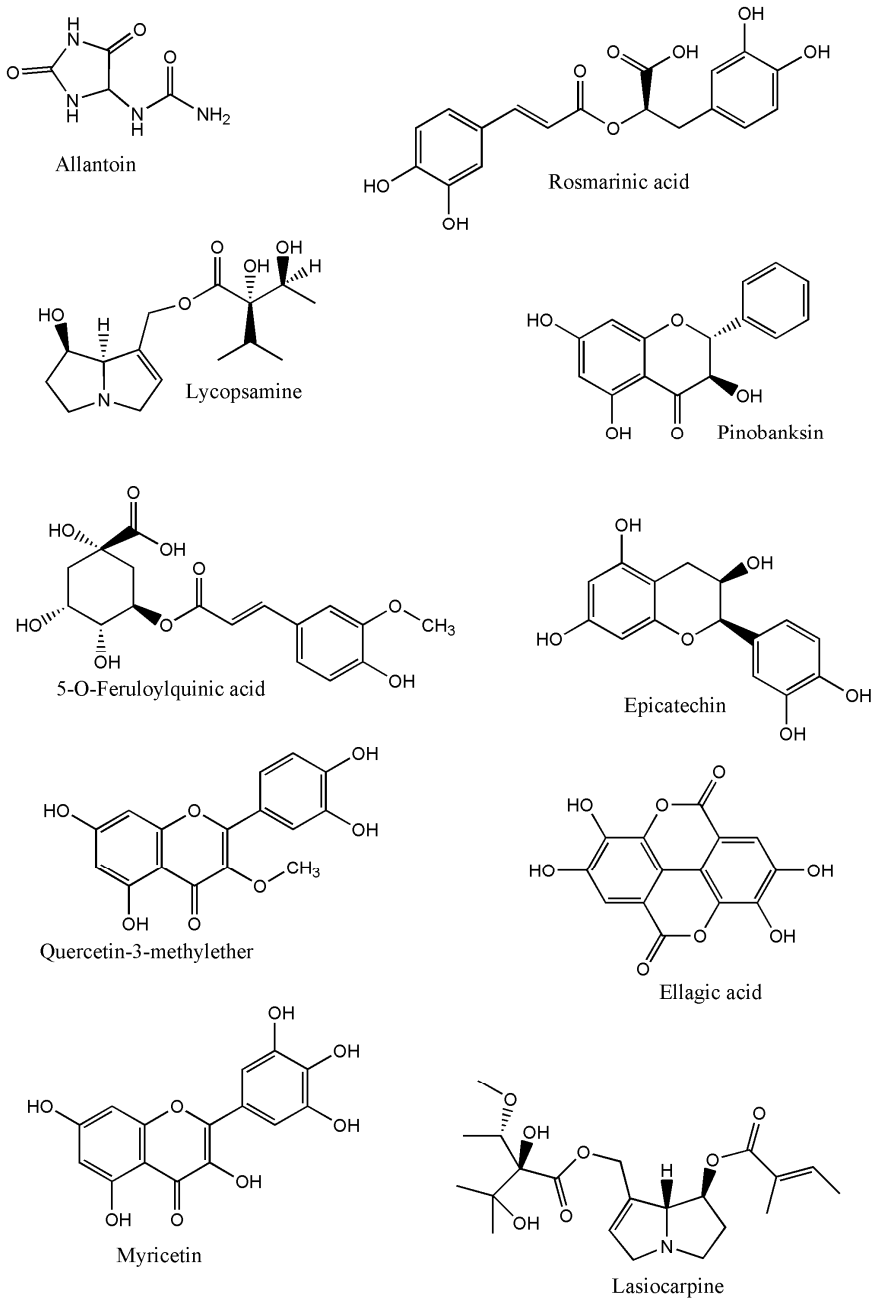


*Figure 2. Structural formulas of the identified compounds in the aqueous extract of comfrey root.*

indicates that peak 12 is lasiocarpine. Based on previous studies, it is known that lycopsamine and lasiocarpine belong to the group of pyrrolizidine alkaloids which are of special interest because several of them showed to cause toxic reactions in humans, primarily a veno-occlusive liver disease, when ingested as food or herbal medicines.

**Quantification of bioactive compounds in the aqueous extract of comfrey root**

The results of the qualitative analysis of the aqueous extract of comfrey root showed that allantoin and rosmarinic acid were present in the extract. Besides these two compounds, the presence of ellagic acid was also detected. Due to the biological activity of these three compounds and their role in the total beneficial activity of comfrey, quantitative contents were determined. In Table 2, the obtained results from the quantitative determination of allantoin, ellagic acid and rosmarinic acid are shown. The contents of these compounds were determined by UHPLC–DAD–HESI–MS method using the calibration curves which were constructed for the appropriate bioactive component at 360 nm.

Allantoin is one of the main components responsible for beneficial bioactive properties of comfrey.

*Table 2. The yields of the total extractive matter and the content of allantoin, ellagic acid and rosmarinic acid in the aqueous extract of comfrey root*

| Compound | Regression equation[a] | $R^2$ | g/100 g of dry extract | Total extractive matter (g/100 g of plant material) |
|---|---|---|---|---|
| Allantoin | $y = 12298.88x + 12.86$ | 0.9996 | 8.91 | 6.30 |
| Ellagic acid | $y = 368210x + 110.15$ | 0.9969 | 7.4 | |
| Rosmarinic acid | $y = 8514.7x + 1214.3$ | 0.9983 | 12.8 | |

[a]$y = ax+b$; where $x$ is the concentration in mg/mL, and $y$ is the area under the curve at the selected wavelength

From literature data, it is known that allantoin is present from 0.6–4.7% in comfrey [28]. The results of our study showed that the aqueous extract of comfrey root contains allantoin 8.91 g/100 g of the dry extract (*i.e*., 8.91%) [5]. Based on this it can be concluded that comfrey root represents a good source for the isolation of allantoin.

Literature data indicate that ellagic acid was mostly present in strawberry (162 mg/100 g dry matter) [29] and raspberry fruits (415 mg/100 g dry matter) [30]. In the aqueous extract of comfrey root, the content of ellagic acid is 7.4 g/100 g of the dry extract. The high content of ellagic acid in the aqueous extract of comfrey root can be responsible for its biological activity. Rosmarinic acid was also present in many plant materials, as are the leaves of Lemon balm (*Melissa officinalis*, Lamiaceae) in the concentration of 3.91% [31]. In *Rosmarinus officinalis* (Lamiaceae) rosmarinic acid was detected in leaves, flowers, stems and roots, but the highest amount of 2.5% was found during the first stages of the leaf growth [32]. In the crude extract of *Borago officinalis* (*Boraginaceae*) rosmarinic acid was present with 2.5% [33]. In these studies, the determined content was 12.8%, and to our knowledge this is the highest concentration of rosmarinic acid that has been found in all determined species so far.

**Microbial activity of allantoin and the aqueous comfrey root extract**

The presence of the identified compounds and the high content of allantoin, ellagic acid and rosmarinic acid in the aqueous extract of comfrey root, and the lack of the published results of the antimicrobial activity of comfrey, were a good motive for this study. The results of the microbial activity of the aqueous extract of comfrey root and allantoin are shown in Table 3.

*Table 3. The microbial activity of allantoin and the aqueous extract of comfrey root*

| Microorganism | Tested sample | | | | Positive control | | | | Negative control | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Allantoin ($c_0$ = 10 mg/mL) | | Aqueous extract of comfrey ($c_0$ = 40 mg/mL) | | Doxycycline µg/mL | | Nystatin µg/mL | | Sterilised aqua destilata | |
| | *MIC* | *MBC* | *MIC* | *MBC* | *MIC* | *MBC* | *MIC* | *MBC* | *MIC* | *MBC/MFC* |
| Bacterial strains | | | | | | | | | | |
| *B. subtilis* (ATCC 6633) | – | – | – | – | 1.56 | 1.56 | nt[a] | nt[a] | na[b] | na[b] |
| *E. coli* (ATCC 8739) | – | – | 10 | 40 | 0.78 | 0.78 | nt[a] | nt[a] | na[b] | na[b] |
| *P. aeruginosa* (ATCC 27857) | – | – | – | – | 12.5 | 12.5 | nt[a] | nt[a] | na[b] | na[b] |
| *S. typhimurium* (ATCC 14028) | – | – | 10 | 20 | 6.25 | <50.0 | nt[a] | nt[a] | na[b] | na[b] |
| *S. aureus* (ATCC 6538) | – | – | – | – | 6.25 | 0.78 | nt[a] | nt[a] | na[b] | na[b] |
| Fungi | | | | | | | | | | |
| *A. niger* (ATCC 16404) | – | – | – | – | nt[1] | nt[a] | 0.78 | 0.78 | na[b] | na[b] |
| *C. albicans* (ATCC 10231) | – | – | – | – | nt[1] | nt[a] | 6.25 | 6.25 | na[b] | na[b] |

[a]Standard reference of the sample that is not tested; [b]standard reference of the tested sample that did not show activity

Based on the obtained results, it can be concluded that *Escherichia coli* ATCC8739 and *Salmonela typhimirium* ATCC6538 were the most sensitive to the aqueous extract of comfrey root but, on the other hand, the aqueous extract of comfrey root did not show the activity on the investigated fungi strains. Doxycycline, a commercial antibiotic which was used as the control sample showed the antimicrobial activity on all the investigated bacteria species. The results of the microbial activity of allantoin showed that allantoin did not express the antimicrobial activity on all the investigated bacteria species. Based on this, it can be concluded that allantoin is not responsible for the antimicrobial activity of the aqueous extract of comfrey root.

## CONCLUSIONS

By using a new UHPLC–DAD–HESI–MS method 12 compounds were separated and identified in the aqueous extract of comfrey root. The highest content of the identified components was determined for rosmarinic acid (12.8%), allantoin (8.91%) and ellagic acid (7.4%). The aqueous extract of comfrey root showed the best microbial activity against *Escherichia coli* ATCC8739 and *Salmonela typhimirium* ATCC6538, while the standard of allantoin did not show activity against any of the examined microbe strains. This indicates that allantoin is not responsible for the antimicrobial activity of the aqueous extract of comfrey root. The obtained results could be beneficial for the potential application of the aqueous extract of comfrey root as a source of bioactive components in pharmaceutical and cosmetic industry.

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## IZVOD

### IDENTIFIKACIJA I KVANTIFIKACIJA BIOAKTIVNIH JEDINJENJA IZ VODENOG EKSTRAKTA KORENA GAVEZA POMOĆU UHPLC–DAD–HESI–MS METODE I NJEGOVA MIKROBIOLOŠKA AKTIVNOST

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U ovom radu ispitivan je kvalitativni i kvantitativni sastav vodenog ekstrakta iz korena gaveza (*Symphytum officinale* L.) dobijen metodom po Soxhletu. Pored kvalitativne i kvantitativne analize ispitivana je i upoređena antimikrobna aktivnost alantoina i vodenog ekstrakta korena gaveza. Primenom nove UHPLC–DAD–HESI–MS metode identifikovano je 12 jedinjenja. Visok sadržaj alantoina, elaginske kiseline i ruzmarinske kiseline (8.91, 7.4 i 12.8%, redom) u vodenom ekstraktu korena gaveza ukazuje na činjenicu da se koren gaveza može koristiti kao izvor za njihovo dobijanje. Prisustvo bioaktivnih jedinjenja u vodenom ekstraktu korena gaveza dalo je dobru osnovu za ispitivanje i mikrobiološke aktivnosti. Rezultati ispitivanja antimikrobne aktivnosti su pokazali da su *Escherichia coli* ATCC 8739 i *Salmonela typhimirium* ATCC 6538 najviše osetljive na vodeni ekstrakt iz korena gaveza. Međutim, s druge strane vodeni ekstrakt iz korena gaveza nije pokazao mirobiološku aktivnost na ispitivane sojeve gljivica. Pored vodenog ekstrakta korena gaveza ispitivana je i mikrobiološka aktivnost alantoina, kao važnog konstituenta korena gaveza. Iz dobijenih rezultata se vidi da alantoin nije pokazao aktivnost na ispitivane sojeve mikroba. Na osnovu dobijenih rezultata može se zaključiti da alantoin nije odgovoran za mikrobiološku aktivnost ekstrakta korena gaveza.

*Ključne reči*: Koren gaveza • *Symphytum officinale* L. • UHPLC–DAD–HESI–MS • Mikrobiološka aktivnost