Anti-\textit{Acinetobacter baumannii} activity of \textit{Rumex crispus} L. and \textit{Rumex sanguineus} L. extracts

Verica Aleksic Sabo\textsuperscript{1}, Emilija Svircev\textsuperscript{2}, Neda Mimica-Dukic\textsuperscript{2}, Dejan Orcic\textsuperscript{2}, Jelena Narancic\textsuperscript{1}, Petar Knezevic\textsuperscript{1,2,}\textsuperscript{*}

\textsuperscript{1}Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovica 3, 21 000 Novi Sad, Vojvodina, Serbia
\textsuperscript{2}Department of Chemistry, Biochemistry and environmental protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovica 3, 21 000 Novi Sad, Vojvodina, Serbia

\textbf{ABSTRACT}

\textbf{Objective:} To examine the effect of \textit{Rumex crispus} (\textit{R. crispus}) and \textit{Rumex sanguineus} (\textit{R. sanguineus}) plant extracts against isolates of \textit{Acinetobacter baumannii} (\textit{A. baumannii}) from wounds, including multidrug-resistant strains.

\textbf{Methods:} Six prepared \textit{Rumex} extracts were subjected to liquid chromatography-tandem mass spectrometry. Antimicrobial activity of extracts and pure compounds (catechin, quercetin, isorhamnetin, emodin, and gallic acid) was examined by a microtiter plate method, while for determination of compound binary combinations activity a checkerboard method was applied. Active fractions of extracts were detected by agar-overlay high-performance thin-layer chromatography-bioautography assay followed by liquid chromatography - diode array detection - mass spectrometry analysis.

\textbf{Results:} A total of 28 compounds were detected in two extracts of \textit{R. crispus} and 26 compounds in four different \textit{R. sanguineus} extracts, with catechin as a dominant component. Anti-\textit{A. baumannii} activity was confirmed for all six \textit{R. sanguineus} and \textit{R. crispus} extracts at the concentration range from 1 to 4 mg/mL. Neither examined single compounds nor their binary combinations exhibited an anti-\textit{A. baumannii} activity (MIC>256 \textmu g/mL). The bioautography showed that fractions with the most prominent anti-\textit{A. baumannii} activity tended to contain more polar compounds, predominantly flavonol (quercetin and kaempferol) glycosides; but also fractions containing flavanone (eriodictyol) glycosides and anthraquinone (emodin) glycosides; and less polar eriodictyol aglycone.

\textbf{Conclusions:} The results justify and elucidate the traditional application of \textit{R. sanguineus} and \textit{R. crispus} extracts for wound healing, indicating the necessity for their further examination in combat against multidrug-resistant \textit{A. baumannii} isolates from wounds.

\textbf{KEYWORDS:} \textit{Acinetobacter baumannii}; Isolates; Wound; Multidrug resistance; \textit{Rumex} extracts; High-performance thin-layer chromatography-bioautography

\section*{1. Introduction}

\textit{Acinetobacter baumannii} (\textit{A. baumannii}) is a Gram-negative coccobacillus that colonizes the oral cavity, respiratory and gastrointestinal tract, but is also a recognized opportunistic pathogen that causes various severe infections. \textit{A. baumannii} is a frequent cause of wound infections, particularly war-related injuries, because of easy wound/burn contamination. On the other hand, it is a clinically dominant species with a pronounced tendency to induce nosocomial infections, especially in intensive care units\cite{1,2}. The infections with the etiological agent as \textit{A. baumannii} have a high mortality risk\cite{3,4}.

This genomic species is the most troublesome member of \textit{Acinetobacter calcocaceticus-A. baumannii} complex (Acb-complex), showing pronounced resistance to conventional antibiotics. In the early 1970s, infections caused by this bacterium were treated with gentamicin, minocycline, nalidixic acid, ampicillin, and...
carbenicillin. However, between 1971 and 1974, this bacterium became resistant to the aforementioned antimicrobials. During the early 1990s, the bacterium has exhibited resistance to beta-lactams[5], aminoglycosides[6], chloramphenicol, tetracycline, and fluoroquinolones[7]. At the end of the 1990s, carbapenems were the only treatment choice[5], and so rifampicin was introduced in combination with carbapenems. To combat multi-drug resistant strains, tigecycline, polymyxin B, and colistin are used nowadays. However, resistance to these antibiotics has also been demonstrated recently, which makes this bacterium one of the greatest threats to human health today. The emergence of _A. baumannii_ strains resistant to all known antibiotics, _i.e._ pandrug resistant strains indicates urgent necessity to discover novel antimicrobial agents or therapeutic strategies[8]. Natural plant products, particularly those used in traditional medicine have become the solutions to overcome this problem[9–11]. Many studies have proved the effectiveness of nature-derived antimicrobial agents in the treatment of various diseases and support their usage to a great extent nowadays.

Besides the increasing emergence and spread of multi-drug resistant and pandrug resistant microorganisms, environmental awareness among the population is the reason for the current application of natural products. The use of natural antimicrobial agents as phytopharmaceuticals and food preservatives is an increasingly accepted alternative to the synthetic and usually toxic, teratogenic or mutagenic chemicals. From 2000 to 2006, about 50% of new molecules were extracted from natural products, indicating their importance in the development of new drugs for the treatment of infectious diseases[12]. Thus, in comparison to conventional antibiotics, natural antimicrobial agents have many advantages, such as less harm, broad acceptance because of their traditional use, better biodegradation, less bacterial resistance, etc.

_Rumex crispus_ (R. crispus) L. and _Rumex sanguineus_ (R. sanguineus) L. from the Polygonaceae family have been used in ethnopharmacology for various medical purposes, and some of the applications indicate their antimicrobial activity and/or wound healing potential[13]. For instance, _R. crispus_ has been used in Hungary and in Romania for diarrhea, rashes, sores and wounds treatments[14,15], while the dried underground plant parts have been used in traditional Turkish medicine as a blood cleanser[16]. In other parts of the world, it has also been used against skin diseases and against dysentery[17]. _R. crispus_ has been applied to India and Pakistan for treating a wide range of skin problems (sores, ulcers, and wounds) and the underground parts have been used in diarrhea treatment[18,19]. Similarly, _R. crispus_ was used by American Indian tribes for the treatment of diarrhea, dysentery and skin problems[20], including fungal infections[21]. The infusion or decoction of _R. crispus_ has been commonly used in folk medicines by natives of Africa for the treatment of helminths, wounds, internal bleeding and vascular diseases[22]. In Italy, warm leaves of _R. crispus_ and _R. sanguineus_ are applied to treat abscesses. A compress made of half-peeled leaves along with other components has been applied to wounds and sores as the cicatrizing agent. Leaves crushed in mortar were used against abscesses, burns and insect bites[23]. In addition, young leaves of _R. crispus_ that appear in the spring have been used for consumption, while the seeds are collected during the summer and used as an Asian national remedy. It has been reported that _Rumex_ extracts possess antioxidant, antimicrobial and antifungal properties[20], but their activity against _A. baumannii_ has not been examined.

Taking into account the traditional application of _R. crispus_ and _R. sanguineus_ plant extracts in treating wound healing and the necessity to find alternatives to combat multiple drug resistant (MDR) bacteria, the activity of these extracts, their pure compounds individually and in binary combinations against isolates of _A. baumannii_ from wounds was examined. Also, high-performance thin-layer chromatography (HPTLC)-bioautography assay and chemical characterization of the potent extract were performed to elucidate compound(s) responsible for anti-A. baumannii activity.

### 2. Materials and methods

#### 2.1. Standards and reagents

Reference standards of the phenolic compounds were obtained from Sigma–Aldrich Chem (Steinheim, Germany), Fluka Chemie Gmbh (Buchs, Switzerland), Chromadex (Santa Ana, USA), or from Extraysthese (Genay Cedex, France). HPLC gradient grade methanol was purchased from J. T. Baker (Deventer, The Netherlands), and p.a. formic acid and DMSO from Merck (Darmstadt, Germany).

Naturstoff reagent A (diphenylboric acid 2-amino-ethyl ester) was purchased from Roth (Carl Roth GmbH + Co Karlsruhe, Germany), polyethylene glycol 4000 from Sigma–Aldrich (Germany), HPLC grade ethanol from J. T. Baker (Deventer, The Netherlands), toluene, analytical grade from Centrohem (Stara Pazova, Serbia) and ethyl-acetate, analytical grade, from Fischer Company (Fisher Scientific UK Ltd).

#### 2.2. Plant extract preparation

Extracts were prepared using _R. crispus_ (voucher number 2-1721) and _R. sanguineus_ (voucher numbers 2-1735 and 2-1736) from family Polygonaceae. Taxonomic determination of plant material, voucher specimens preparation, and plants samples deposition (at BUNS Herbarium) were done by Goran Anačkov Ph.D., University of Novi Sad Faculty of Sciences, Department of Biology and Ecology. Six extracts in total were prepared for testing the anti-A. baumannii activity (Supplementary Table 1). All the extracts were prepared by maceration of dry, grounded plant material (above ground plant parts – for the herb extracts; and the underground parts – for the rhizome extracts) with 80% ethanol at constant shaking for 48 h. Filtered extracts were evaporated in vacuum and re-dissolved...
in 70% ethanol (for microbiological analysis) or dimethyl sulfoxide (DMSO) (for chemical analysis), giving a final concentration of 200 mg/mL or 300 mg/mL. Herb extracts of Rumex species were washed (liquid-liquid extraction) with petroleum ether to remove lipids and pigments. Washed extracts were concentrated in vacuum and re-dissolved in DMSO or 70% ethanol giving a final concentration of around 200 mg/mL or 300 mg/mL.

2.3. Bacterial strains

A total of 25 bacterial strains were used in the study. Three reference strains of A. baumannii were used, two from American Type Culture Collection (ATCC 19606 and ATCC BAA747, Rockville, MD, USA) and one from the National Collection of Type Cultures (NCTC 13420, Public Health England, UK). In addition, two reference strains, Escherichia coli (E. coli) ATCC 25922 and Staphylococcus aureus (S. aureus) ATCC 25923, were used as quality controls. The twenty remaining strains were MDR A. baumannii isolates from outpatient and clinical wounds, which have been characterized previously [16]. All the bacterial strains were stored in Luria Bertani broth supplemented with glycerol (10% v/v) at −70°C. All antibacterial tests were performed using Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB).

2.4. Antibacterial activity determination

2.4.1. Effect of plant extracts

In order to find the alternative solution(s) for the eradication of A. baumannii, the minimal inhibitory concentrations (MICs) of extracts were examined by a slightly modified microtitre plate method [24]. In the 96-well microtiter plates, double dilutions of extracts were prepared in sterile distilled water and the final concentrations of each extract in the microtiter plate ranged from 0.25 to 8 mg/mL. The final concentrations of the plant extract solvents did not exceed 1.9% for ethanol extracts and 1% for the extracts prepared in DMSO. All experiments included the control of the maximum solvent concentration in the final volume to confirm the absence of inhibition. The final bacterial count in the test was approximate 1×10⁶ CFU/mL. Reference strains E. coli ATCC 25922, S. aureus ATCC 25923 and gentamicin were used as method quality controls. Microtiter plates were incubated overnight at 37°C, after which 10 μL of 1% triphenyl-tetrazolium chloride (TTC) solution was added to each well and the microtiter plates were additionally incubated for 2 h at 37°C until red color of formazan appeared. This modification was made in order to make MIC values determination more precise. The minimum concentration of extracts that prevented the appearance of red color, i.e. formation of formazan was considered as a MIC value.

Minimal bactericidal concentration (MBC) was determined by spreading 10 μL of the suspension from wells without obvious bacterial growth onto MHA, in order to determine if the type of the bacterial inhibition was permanent or reversible. Plates were incubated for 24 h at 37°C. After the incubation, the presence or absence of growth was recorded, where the lowest plant extract concentration at which the bacterial cell count was reduced by 99.9% compared to the initial number, was considered as MBC.

2.4.2. Effect of selected extract compounds

In order to determine anti-A. baumannii activity of components from plant extracts, some dominant compounds from extracts and those detected in the R. crispus herb extract after chemical characterization were tested. The following five phenolic compounds were tested: quercetin, quercetin-3-O-glucoside, catechin, emodin and gallic acid.

Anti-A. baumannii effect of standard compounds was tested using the same method as described for herbal extracts. Compounds were diluted depending on their solubility in water, methanol or DMSO (not exceeding 1%) to the final tested concentrations ranging from 0.125 to 256 μg/mL.

2.4.3. Effect of extract compounds in binary combinations

The anti-A. baumannii effect of selected compounds in the examined extracts was tested after the preparation of different binary combinations (1:1, v/v). This method was used due to high MIC values (>256 μg/mL) and poor antibacterial activity of a single compound. For each combination, double dilutions were prepared so that the concentrations of bioactive compounds varied from 16 to 128 μg/mL. The incubation period, data interpretation and presentation of obtained results were performed as described for herbal extracts and individual bioactive components.

2.5. Agar-overlay HPTLC–bioautography assay

The method that combines microbiological assay with the thin-layer chromatography [14,15] was used for further analysis of anti-A. baumannii active substances. In this test, the focus went towards Rumex species which showed the best activity (i.e. lowest MIC and MBC values).

Selected extract separation was performed on HPTLC silica gel 60 F254 aluminum plates (Merck, Germany), measuring 10 cm×20 cm. R. crispus 171_H extract was applied to the plate in the form of a narrow band (5 μL, i.e. 1 mg/mL). Four such bands were applied on one piece of plate. After drying the samples, the plate was developed with the previously optimised mobile phase (ethyl acetate-toluene-formic acid-water 80:10:5:5, v/v/v/v). The development mode was ascendant, in a saturated chamber. After chromatographic separation, the adsorbent layers were dried in an oven at 90°C for 5 min to remove the solvent completely. Methanolic Naturstoff reagent A, NA (1.0%) and ethanolic polyethylene glycol 4000 solution (5.0%) were used to visualize the separated compounds. The sprayed plates were
observed under VIS and UV light (366 nm) and documented. Separation of the extract components was done in quadruplicate, with two bands used for the bioautographic analysis of plant extract, one band used for visualization of separated components, and one for the extraction of spotted fractions (zones) and further liquid chromatography with diode array detection and mass spectrometry (LC/DAD/MS) analysis (as described below). Fractions on the HPTLC plates were spotted in accordance with the results of the bioautography test.

For the agar-overlay bioautography assay, two unsprayed HPTLC plate bands were cut out after extract separation. One strip was used as a whole, but the other was chopped into squares based on the difference in colour obtained on the third strip. The prepared parts of the HPTLC plates were positioned on the surface of the MHA and then topped with an inoculated semisolid medium (a bacterial suspension approximate 1.5x10⁶ CFU/mL) supplied with 1% TTC solution (final ratio 30:1:1, v/v/v). The plates were incubated for 24 h at 37°C. After incubation, the absence of bacterial growth indicated the zones with antimicrobial activities. The TTC was used to facilitate the visualization of the presence/absence of bacterial growth[25]. Inhibition zones are visible as transparent zones against red-colored bacterial growth. The HPTLC plate strips were photographed using the Canon EOS 100D camera.

2.6. Extracts’ chemical composition analysis

All the measurements were done using Agilent Technologies 1200 Series High-performance liquid chromatography coupled with Agilent Technologies 6410A Triple Quad tandem mass spectrometer with electrospray ion source and controlled by Agilent Technologies MassHunter Workstation software (ver. B.03.01).

2.6.1. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis of plant extracts

For the quantitative LC-MS/MS analysis of the selected 45 compounds, extracts were diluted with 0.05% aqueous formic acid and methanol (1:1) to a final concentration of 2 mg/mL. The method used in this study for the Rumex extracts analysis was previously developed, validated and published by Orčić et al[26].

2.6.2. LC/DAD/MS analysis of R. crispus herb extract

Following the results of the bioautography test, eight different zones were spotted on the HPTLC plate. Every zone was scraped from aluminum sheet, and compounds were extracted from the silica gel with 80% methanol (400 μL), and filtered through syringe filters, regenerated cellulose, 0.45 μm into vials. A total of 5 μL of these samples were injected into the system, with Zorbax Eclipse XDB-C18 (50 mmx4.6 mmx1.8 μm) rapid resolution column held at 50°C. The mobile phase was delivered at a flow rate of 0.8 mL/min in a gradient mode (0 min 20% B, 6.67 min 60% B, 8.33 min 100% B, 12.5 min 100% B, re-equilibration time 4 min). Eluted components were firstly recorded on diode array detector, full spectra in 190-700 nm range, but also detected by MS, using the ion source parameters as follows: nebulization gas (N₂) pressure 40 psi, drying gas (N₂) flow 9 L/min and temperature 350°C, capillary voltage 4 kV; and using MS2Scan run mode (negative and positive ionization, NI/PI, m/z range of 120–1000 and fragmentor voltage of 80 V). For the one dominant compound in fraction 7 (F7), a product ion scan experiment was conducted using collisionally induced dissociation (high-purity N₂ as the collision gas, collision energies ranging 10–40 V in 10-V increments).

2.7. Statistical analysis

The MICs were logarithmically transformed and data were tested for normality of distribution. Because of the lack of normal distribution, differences in extract activity and differences in MIC and MBC among different plant species and plant parts were estimated by the Wilcoxon signed-rank test. The level of significance for all analyses was set as α=0.05.

All the experiments were performed in triplicates and on three independent occasions and the results are represented as geometric means of replications.

3. Results

3.1. Anti–A. baumannii effect of R. sanguineus and R. crispus

The extracts of R. sanguineus and R. crispus showed significant bacteriostatic and bactericidal activity against MDR A. baumannii isolates from wounds (Table 1).

The extracts of R. sanguineus from the Zrnajevac on Fruška Gora Mountain (4NZ_H_p and 4NZ_R) exhibited bacteriostatic activity with the MIC ranging 1.0–2.8 mg/mL for the extract of plant aerial parts (herb) and 1.4–4.0 mg/mL for the extract of the underground plant parts (rhizome). The bactericidal effect of these extracts showed the same MBC values (2.0–5.7 mg/mL) regardless of the part of the plant. Extracts of the same plant species originating from the Ćirki venac on Fruška Gora Mountain (4Z_H_p and 4Z_R) also exhibited a bacteriostatic and bactericidal effect, with MIC values of 1.0–2.0 mg/mL for herb extract, or 1.4–2.8 mg/mL for the extract of rhizomes, while MBC values varied in the range 1.0–4.0 mg/mL for herb extract, or 1.4–5.7 mg/mL for rhizomes extract. The differences in MIC and MBC values among different R. sanguineus extracts were significant (P<0.001). Considering the different parts of R. sanguineus, herb extracts exhibited a better antibacterial effect, with lower MICs compared to rhizome extracts (P=0.002 for 4NZ_...
Table 1. MICs and MBCs of *Rumex sanguineus*, *Rumex crispus* extracts, and compounds alone and in binary combinations against *Acinetobacter baumannii* wound isolates (mg/mL).

| Acinetobacter baumannii strain | Resistotype | 4NZ_H_p | 4NZ_R | 4Z_H_p | 4Z_R | 171_H | 179_R | Compounds^c^ and binary combinations^c^ | MIC | MBC |
|-------------------------------|-------------|---------|-------|--------|------|-------|-------|----------------------------------------|-----|-----|
| ATCC 19066                    | CRO-CHL-GEN | 1.4     | 2.0   | 1.4    | 2.0  | 1.4   | 2.0   | >0.256                                 |     |     |
| ATCC BA A747                  | CRO-CHL-TET | 1.4     | 2.0   | 1.4    | 2.0  | 1.4   | 2.0   | >0.256                                 |     |     |
| NCTC 13423                    | N.A.        | 2.0     | 2.0   | 4.0    | 5.7  | 2.0   | 2.0   | >0.256                                 |     |     |
| Aba-2572                      | CRO-CHL-GEN-KAN-PMB-TET | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-2793                      | CRO-CHL-GEN-KAN-TET | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-4156                      | AMK-CRO-CIP-CHL-GEN-KAN-PMB-TET | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-4727                      | CRO-CIP-CHL-GEN-KAN-TET | 1.4 | 2.0 | 2.0 | 2.0 | 1.4 | 2.0 | >0.256 |     |     |
| Aba-4779                      | CRO-CIP-CHL-KAN-TET | 1.4 | 2.0 | 2.0 | 2.0 | 1.4 | 2.0 | >0.256 |     |     |
| Aba-4803                      | AMK-CRO-CIP-CHL-GEN-KAN-PMB-TET | 2.0 | 1.0 | 2.0 | 1.0 | 1.4 | 1.4 | >0.256 |     |     |
| Aba-4804                      | AMK-CRO-CIP-CHL-GEN-KAN-PMB-TET | 1.2 | 1.0 | 0.5 | 0.5 | 1.0 | 1.0 | >0.256 |     |     |
| Aba-4890                      | AMK-CRO-CIP-CHL-GEN-KAN-PMB-TET | 1.4 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-4914                      | AMK-CRO-CIP-CHL-GEN-KAN-PMB-TET | 2.0 | 2.0 | 1.4 | 1.4 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-5055                      | CRO-CIP-CHL-GEN-KAN-PMB-TET | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | >0.256 |     |     |
| Aba-5074                      | AMK-CRO-CIP-CHL-GEN-KAN-PMB-TET | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-5081                      | CRO-CIP-CHL-GEN-KAN-PMB-TET | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-5372                      | CRO-CHL-KAN-TET | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-6673                      | CRO-CHL-GEN-KAN-PMB-TET | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-7860                      | CRO-CHL-GEN-KAN-PMB-TET | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-8255                      | CRO-CHL-KAN-TET | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-8781                      | CRO-CHL-KAN | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-8833                      | CRO-CHL-KAN | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-34963                     | CRO-CHL-GEN-KAN-PMB-TET | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| Aba-40100                     | CRO-CHL-GEN-KAN-PMB-TET | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | >0.256 |     |     |
| *Escherichia coli* ATCC 25922^d^ | CRO-CHL-KAN | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | >0.256 |     |     |
| *Staphylococcus aureus* ATCC 25923^d^ | CRO-CHL-KAN | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | >0.256 |     |     |

MIC: Minimal inhibitory concentration; MBC: Minimum bactericidal concentration and they were expressed as geometric mean of the values obtained in three independent repetitions; ^1^ Based on MIC values from ref. 11; N.A. not available; ^2^ Quercetin, isoquercitrin, catechin, emodin, gallic acid; ^3^ binary combinations were: quercetin-isoquercitrin, quercetin-catechin, quercetin-emodin, quercetin-gallic acid, isoquercitrin-catechin, isoquercitrin-emodin, isoquercetin-catechin, isoquercetin-gallic acid, and emodin-gallic acid; ^4^ MIC/MBC for a control antibiotic, *i.e.*, gentamicin was 0.5/1.0 μg/mL for *Escherichia coli* and 0.5/2.0 μg/mL for *Staphylococcus aureus*. H: plant aerial parts (herb) R: underground plant parts (rhizome).
The preliminary results of LC-MS/MS analysis showed that quantitative analysis of the compounds in Rumex plant extracts by LC-MS/MS showed a total of 30 compounds were detected in the extracts of both Rumex species and the presence of 26 compounds in four different Rumex extracts was confirmed. For extracts of Rumex sanguineus, the present phenolic compounds can be classified in different groups as follows: phenyl-carboxylic acids (p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid, gallic acid, caffeic acid, ferulic acid, syringic acid, and 5-O-cafeoylquinic acid); flavonoids (apigenin); flavonols and their glycosides (kaempferol, kaempferol-3-O-glucoside, quercetin, quercitin, quercitin-3-O-glucoside, rutin); flavonoids (naringenin); flavane-3-ols (catechin and epicatechin); and quinic acid. Of the compounds in the Rumex sanguineus extracts, catachin was dominant in amounts 12.31-16.63 mg/g of dry extract in the herb, and 3.49-3.60 mg/g of dry extract in the rhizome. In the herb extracts, it was followed by naringenin (0.63-0.68 mg/g of dry extract) and gallic acid (0.57-0.66 mg/g of dry extract in the herb extracts), and followed by epicatechin in the rhizome extracts (0.66-0.77 mg/g of dry extract).

In Rumex crispus extracts, 28 compounds were confirmed (Table 2), and can be classified in different groups as follows: flavonoid glycosides (apigenin, epicatechin, catechin, luteolin, myricetin, quercetin, naringenin) and methylated derivatives (isorhamnetin and chrysophanol); flavonoid glycosides: 3-O-glycoside (quercetin-3-O-glycoside, kaempferol-3-O-glucoside, and quercitrin), 7-O-glucoside 4NZ_H_p 4NZ_R 4Z_H_p 4Z_R 171_H 179_R

### Table 2. Results of quantitative (LC-MS/MS) analysis of the phenolic compounds in Rumex plant extracts (mg of compound/g of dry extract).

| No. | LQ* | [M-H] | Compounds | Rumex sanguineus extracts | Rumex crispus extracts |
|-----|-----|-------|-----------|--------------------------|-----------------------|
|     |     |       |           | 4NZ_H | 4NZ_R | 4Z_H | 4Z_R | 171_H | 179_R |
| 1   | 0.007 | 191- | Quinic acid | 0.231 | 0.026 | 0.437 | 0.056 | 0.710 | 0.057 |
| 2   | 0.010 | 169- | Gallic acid | 0.573 | 0.316 | 0.661 | 0.229 | 0.949 | 0.251 |
| 3   | 0.002 | 153- | Protocatechuic acid | 0.089 | 0.015 | 0.141 | 0.012 | 0.145 | 0.019 |
| 4   | 0.023 | 289- | Catechin | 12.310 | 3.604 | 16.631 | 3.487 | 41.957 | 13.248 |
| 5   | 0.004 | 353- | 5-O-Caffeoylquinic acid | 0.019 | <LQ | 0.037 | <LQ | <LQ | n.d. |
| 6   | 0.049 | 457- | Epigallocatechin gallate | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ |
| 7   | 0.004 | 137- | p-Hydroxybenzoic acid | 0.021 | 0.005 | 0.010 | 0.007 | 0.015 | 0.009 |
| 8   | 0.031 | 289- | Epicatechin | 0.328 | 0.766 | 0.384 | 0.658 | n.d. | 1.688 |
| 9   | 0.003 | 179- | Caffeic acid | 0.041 | 0.012 | 0.022 | 0.021 | 0.032 | 0.020 |
| 10  | 0.020 | 197- | Syringic acid | 0.037 | 0.054 | 0.024 | 0.024 | 0.041 | 0.092 |
| 11  | 0.002 | 163- | p-Coumaric acid | 0.019 | 0.008 | 0.014 | 0.009 | 0.038 | 0.027 |
| 12  | 0.007 | 193- | Ferulic acid | 0.022 | 0.019 | 0.010 | 0.030 | 0.054 | 0.024 |
| 13  | 0.002 | 431- | Vitexin (apigenin-8-C-glucoside) | <LQ | <LQ | <LQ | <LQ | <LQ | n.d. |
| 14  | 0.002 | 447- | Luteolin-7-O-glucoside | <LQ | <LQ | <LQ | <LQ | <LQ | n.d. |
| 15  | 0.003 | 463- | Hyperoside | n.d. | n.d. | n.d. | n.d. | n.d. | <LQ |
| 16  | 0.002 | 609- | Rutin | 0.048 | 0.009 | 0.034 | 0.010 | 0.114 | 0.021 |
| 17  | 0.002 | 463- | Quercetin-3-O-glucoside | 0.237 | 0.019 | 0.243 | 0.018 | 1.681 | 0.091 |
| 18  | 0.003 | 431- | Apigenin-7-O-glucoside | <LQ | <LQ | <LQ | <LQ | n.d. | n.d. |
| 19  | 0.049 | 317- | Myricetin | <LQ | <LQ | <LQ | <LQ | n.d. | n.d. |
| 20  | 0.001 | 447- | Quercitin (quercetin-3-O-rhamnoside) | 0.044 | 0.035 | 0.041 | 0.030 | 0.074 | 0.190 |
| 21  | 0.002 | 447- | Kaempferol-3-O-glucoside | 0.293 | <LQ | 0.203 | 0.001 | 0.972 | <LQ |
| 22  | 0.049 | 301- | Quercetin | <LQ | 0.064 | <LQ | <LQ | 0.247 | <LQ |
| 23  | 0.003 | 271- | Naringenin | 0.683 | 0.082 | 0.634 | 0.075 | 0.442 | 0.203 |
| 24  | 0.002 | 285- | Luteolin | <LQ | <LQ | <LQ | <LQ | 0.443 | 0.004 |
| 25  | 0.005 | 269- | Apigenin | <LQ | <LQ | 0.006 | <LQ | 0.064 | 0.010 |
| 26  | 0.003 | 285- | Kaempferol | 0.098 | 0.001 | 0.131 | 0.001 | 0.071 | n.d. |
| 27  | 0.013 | 315- | Isorhamnetin | <LQ | <LQ | <LQ | <LQ | 0.006 | n.d. |
| 28  | 0.006 | 269- | Aloe-emodin | n.a. | n.a. | n.a. | n.a. | 0.006 | 0.061 |
| 29  | 0.013 | 293- | Rein | n.a. | n.a. | n.a. | n.a. | n.d. | n.d. |
| 30  | 0.006 | 269- | Emodin | n.a. | n.a. | n.a. | n.a. | 2.601 | 2.271 |
| 31  | 0.003 | 253- | Chrysophanol | n.a. | n.a. | n.a. | n.a. | n.d. | 1.440 |

LQ – limit of quantification; n.d. – not detected; n.a. not analyzed. LC-MS/MS: liquid chromatography-tandem mass spectrometry.
(luteolin-7-O-glucoside and apigenin-7-O-glucoside), 8-C-glycoside (vitexin) and 3-O-ester (epigallocatechin gallate); hydroxybenzoic acids (gallic acid, p-hydroxybenzoic acid, protocatechuic acid, and syringic acid); quinic acid, phenylpropene acids (ferulic acid, caffeic acid, p-coumaric acid) and 5-O-cafeoylquinic acid and anthraquinones (aloemen, emodin, chrysophonol). The most dominant compounds in both R. crispus extracts were catechin (41.96 mg/g of dry herb extract and 13.25 mg/g of dry rhizome extract) and emodin (2.60 mg/g of dry herb extract and 2.27 mg/g of dry rhizome extract).

3.3. Effect of extract compounds alone and in binary combinations

In order to identify the biologically active components responsible for the antimicrobial activity of plant extracts, five standard bioactive components: catechin, quercetin, quercitrin, gallic acid, and emodin were tested. These components were selected according to the criteria for their presence in plant extracts in which antimicrobial activity was detected against the MDR A. baumannii, as well as on the basis of data previously published in the literature.

In all R. sanguineus extracts, catechin was the most dominant component with the amount of 3.487 to 16.631 mg per gram of dry extract (Table 2). The extract of R. sanguineus (4Z_H_p) with the highest catechin content (16.631 mg per gram of dry extract) exhibited the best anti-A. baumannii activity. However, catechin alone did not exhibit antibacterial activity against MDR A. baumannii isolates from wounds, even at the highest tested concentration (256 μg/mL) (Table 1).

### Table 3. Results of LC/DAD/MS analysis of 8 different HPTLC-fractions of Rumex crispus herb extract.

| Fractions | Dominant Rt (min) | Detected compounds | Molecular weight [M-H]⁻/⁺ | NI/PI | Predicted amount (μg) |
|-----------|-------------------|--------------------|----------------------------|---|-----------------|
| F1        | Dark brown 0.70   | Disaccharide       | 377-, 365+                 |     | 0.708           |
|           | 0.71              | Quinic acid        | 190 191-                   |     |                 |
|           | 5.53              | n.i.               | 511-                       |     |                 |
|           | 9.39              | n.i.               | 250-                       |     |                 |
| F2        | Dark orange 0.75  | Quercetin-3-O-glucuronide | 133-, 135+               |     | 0.074           |
|           | 4.47              | n.i.               | 478 477-, 479+            |     |                 |
| F3        | Pale blue 0.75    | Quercetin-3-O-glucoside | 464 463-                 |     | 1.680           |
|           | 4.63              | Quercetin-3-O-derivative | 464 463-                 |     | 1.680           |
| F4        | Orange-brown 0.75 | Eriodictyol-hexoside | 450 449-                  |     | 0.096           |
|           | 5.12              | n.i.               | 462 461-, 463+            |     |                 |
|           | 5.31              | Kaempferol-3-O-glucoside | 448 447-            |     | 0.246           |
|           | 7.56              | Emodin-8-O-glucoside | 432 431-                  |     |                 |
|           | 7.88              | Emodin-8-O-malonyl-hexoside | 518 517-              |     |                 |
| F5        | Yellow 3.27       | Galloyl-catechin    | 442 441-, 443+           |     | 0.145           |
|           | 5.87              | Eriodictyol-rhamnose | 434 433-                  |     |                 |
|           | 6.28              | Emodin-8-O-glucoside | 432 431-                  |     |                 |
|           | 7.62              | n.i.               | 433-, 469-, 491-, 547-, 736- | |                  |
| F6        | Blue-violet-blue 0.95 | Gallic acid | 170 169-                  |     | 0.946           |
|           | 1.66              | Catechin           | 290 289-                  |     | 41.800          |
|           | 1.48              | Protocatechuic acid | 154 153-                |     | 0.145           |
|           | 3.63              | Ferulic acid       | 194 193-                  |     | 0.054           |
| F7        | Light brown 1.49  | Protocatechuic acid | 154 153-                |     | 0.145           |
|           | 2.44              | n.i.               | 172-, 174+                |     |                 |
|           | 3.84              | n.i.               | 187 254-, 256-, 188+, 170+, 210+ | |                  |
|           | 5.79              | Eriodictyol        | 288 287-                  |     |                 |
|           | 6.5               | Quercetin          | 302 301-                  |     | 0.246           |
|           | 6.89              | Luteolin           | 286 285-                  |     | 0.442           |
|           | 7.58              | Kaempherol         | 286 285-                  |     | 0.071           |
|           | 9.27              | Emodin             | 270 269-                  |     | 2.590           |
| F8        | Yellow 2.4        | n.i.               | 236-, 238+, 295-          |     |                 |
|           | 2.42              | n.i.               | 282-, 419+                |     |                 |
|           | 3.85              | n.i.               | 187 186-, 210+, 170+, 210+ | 415+, 432+, 438+ | |
|           | 4.42              | n.i.               | 138-                      |     |                 |
|           | 6.76              | Naringenin         | 272 271-                  |     | 0.441           |
|           | 7.31              | Isorhamnetin       | 316 315-                  |     | 0.006           |
|           | 9.28              | Emodin             | 270 269-                  |     | 2.590           |

n.i. - not identified. The compounds are listed in the order of occurrence in the fractions (zones) of the high-performance thin-layer chromatography (HPTLC) and the amount of the compound in the zone represents the approximate values (based on quantitative analysis of total extract). NI/PI negative /positive ionisation mode. LC-DAD-ESI-MS: liquid chromatography coupled with diode array detector and electrospray ionization mass spectrometry.
The antibacterial activities of quercetin and its derivative quercetin-3-\textit{O}-glucoside (isoquercitrin) as the bioactive components of the flavonoid class were also tested. Quercetin was 0.064 mg/g in \textit{R. sanguineus} (4NZ_R) dry extract (Table 2). Methanol and DMSO solutions of quercetin and water solution of isoquercitrin did not exhibit considerable antibacterial activity against MDR \textit{A. baumannii} isolates since MIC values were greater than 256 μg/mL (Table 1).

Gallic acid, as a representative of hydroxybenzoic acid derivatives, was present in \textit{Rumex} extracts, especially in \textit{R. sanguineus} 4Z_H_p (0.661 mg per gram of dry extract) (Table 2). The results show no activity against MDR \textit{A. baumannii} (Table 1). The lack of activity was also recorded for emodin in the herb and rhizome extracts in the amount of 2.3-2.6 mg per gram of dry extract (Table 2).

In addition, similar to the results of the individual bioactive components of herbal extracts, their binary combinations did not exhibit an antibacterial effect (Table 1).

### 3.4. Agar–overlay HPTLC–bioautography detection coupled with LC/DAD/MS analysis

After the HPTLC separation of \textit{R. crispus} herb extract (sample 171_H), eight different zones of compounds similar in colour were detected (Figure 1B). Some of these fractions (F1-F5 and F7) showed considerable anti-\textit{A. baumannii} activity according to bioautography (Figure 1A). The quantitative LC-MS/MS indicated that this plant is a potentially rich source of biologically active phenolic compounds (Table 3). Using LC/DAD/MS analysis of different fractions, additional compounds were detected: quercetin-3-\textit{O}-glucuronide (F2), eriodictyol (F7) and its derivatives –hexoside (F4) and -rhamnoside (F5), emodin derivatives -8-\textit{O}-glucoside (F4 and F5), and -8-\textit{O}-malonyl hexoside (F4); but also some of the compounds confirmed by LC-MS/MS analysis (e.g. rutin, 5-\textit{O}-caffeoylquinic acid) were not detected in the fractions due to their small amount present on HPTLC or wastage during the process of compounds extraction from the silica gel.

According to the LC/DAD/MS analysis of each fraction, a total of 36 different compounds was detected in \textit{R. crispus} 171_H extract, among which 25 were identified (Table 3). The obtained spectral data were not sufficient for more detailed identification of the remaining 11 compounds. The most abundant compounds were catechin (~41.800 μg) in the zone F6, emodin (~2.590 μg) in F7, and quercetin-3-\textit{O}-glucoside (~1.680 μg) in F2.

The strips were cut according to eight fractions spotted under the UV illumination and bioautography was carried out (Figure 1). The bacterial growth inhibition was obvious on the start of the strip, i.e. in the zone with fractions F1-F5 (R_f<0.4) on the HPTLC strip (Figure 1A). In these zones mostly the flavonoid glycosides were detected while their aglycones had higher R_f values (less polar compounds), located in the upper fractions, near the front of the chromatogram (Figure 1B and C). According to the bioautography assay coupled with LC/MS analysis, the detected compounds with anti-\textit{A. baumannii} activity were mostly in the fractions of quercetin derivatives: quercetin-3-\textit{O}-glucuronide (F2), isoquercitrin (quercetin-3-\textit{O}-glucoside) (F2 and F3), quercetin-3-\textit{O}-derivative (F3), quercitrin (quercetin-3-\textit{O}-rhamnoside) (F3) and quercetin-3-\textit{O}-glucoside (F2 and F3) (Figure 1 and Table 3). The other polyphenols glycosides

![Figure 1](image-url) Bioautogram (A), high-performance thin-layer chromatography chromatogram,detection with NP/PEG reagent under 366 nm (B), and liquid chromatography-diode array-mass spectrometry detection of the compounds in each zone (fraction) (C) of \textit{Rumex crispus} herb extract against \textit{Acinetobacter baumannii} ATCC 19606 strain.
were present in A. baumannii inhibiting fractions: kaempferol-3-O-glucoside (F4), emodin-8-O-glucoside (F4 and F5), emodin-8-O-malonyl-hexoside and eriodictyol-hexoside in F4, eriodictyol-rhamnose and galloyl-catechin in F5. However, the water solution of pure quercetin-3-O-glucoside tested alone did not show antibacterial activity. The predicted amount of quercetin-3-O-glucoside on the HPTLC was 1.681 μg (F3 and F4) (Table 3). Other phenolic glycosides present were not as affordable as the pure compounds for further testing in this study. Similarly, in the fraction F7, which showed bacterial inhibition in bioautography, the quercetin (0.247 μg) and emodin (2.590 μg) were detected and they did not show anti-A. baumannii activity when tested alone. The approximate amount of gallic acid, emodin, and catechin in F6 or F8 was 0.946 μg (F6), 2.590 μg (F8), and 41.800 μg (F6), respectively.

4. Discussion

Extracts of R. sanguineus and R. crispus plants have not been examined previously against A. baumannii, despite their application as traditional wound remedies. In the present study, we confirmed anti-A. baumannii activity of the ethanol extracts. Yildirim et al.[27] also proved that ether and ethanol extracts of R. crispus leaves and seeds showed antibacterial activity in contrast to aqueous extracts. However, they have demonstrated antibacterial activity by disc diffusion method only against bacterial strains S. aureus and Bacillus subtilis with an inhibition zone of 0.8-1.1 cm, whereas strains of Pseudomonas aeruginosa, E. coli, and Candida albicans were resistant to these extracts. In our study, the antibacterial activity of R. crispus extracts was detected against Gram-negative MDR isolates by the microdilution method. It seems that the antibacterial activity of ethanol extracts of herb and rhizomes is better compared with the activity of ether and aqueous extracts tested by Yildirim et al.[27]. Ethanol extracts of R. crispus rhizome showed similar activity against Pseudomonas aeruginosa and E. coli and it was superior to water, acetone, and methanol extracts[28].

The variation in the composition of extracts had an impact on anti-A. baumannii activity, depending on species, plant part, and geographical origin. The effect of geographical variations on the composition of the extract can be overcome by plant growth in a greenhouse under strictly controlled conditions.

All examined extracts showed significant anti-A. baumannii activity against MDR isolates from wounds. These MDR strains could be eradicated by tested Rumex extracts avoiding last line defense antibiotics, such as polymyxins. For these reasons, the extracts should be further examined for its potential topical application in the treatment of A. baumannii infected wounds in vivo.

The selected components of extracts did not show considerable activity against A. baumannii when they were applied as single agents. Catechins are generally considered as efficient antimicrobial agents that exhibit antibacterial activity by inhibiting the N-terminal fragment of DNA gyrase or interacting with its ATP binding site[28]. Catechin is the dominant component of the genus Rumex plants extracts, and its level was significantly higher in herb than in rhizome extracts, which indicates that this component may be responsible for antibacterial activity. However, this activity is not confirmed by the study with catechin activity alone. The antibacterial activity of catechin derivatives, that were not examined in the present study, seems to be more active against Gram-negative bacteria, with MICs in range of 32-512 μg/mL, although some are inactive (MIC>800 μg/mL)[29-31]. Quercetin and quercetin-3-O-glucoside were also inactive against A. baumannii, which is in accordance with the previous report, which found that quercetin-3-O-glucoside was inactive against various bacteria even in extremely high concentration (100 mg/mL)[32,33]. Gallic acid was inactive against A. baumannii at higher concentrations, although it was reported that gallic acid showed MICs against A. baumannii transposon mutant strains as 128-256 μg/mL[34]. The discrepancy is probably due to the difference in the degree of sensitivity/resistance, compared to MDR isolates from wounds in the present study. Similarly, after 24 h of treatment with 200 μg/mL catechin and gallic acid, the number of clinical isolates of MDR A. baumannii belonging to European clones I and II was generally low with 1.2%-9.7% and 4.3%-8.7% of reduction, respectively[35]. When emodin was administered alone, it did not exhibit considerable anti-A. baumannii activity neither, which is in accordance with the previous report, which showed no activity against Gram-negative bacteria Klebsiella pneumoniae and E. coli in emodin (MIC>500 μg/mL)[36].

The activity of the components in binary combinations against A. baumannii was not observed. The synergy between quercetin-epigallocatechin gallate against meticillin-resistant staphylococci has been previously proven[37], as well as between quercetin and gallic acid, p-anisic acid and cinnamic acid against Aeromonas salmonicida, but in all cases, MICs were very high (MIC> 256 μg/mL)[38]. It is a lack of data in the literature on the other combinations tested in this study (quercetin-isouqueritrin, quercetin-catechin, quercetin-emodin, quercetin-gallic acid, isouqueritrin-catechin, isouqueritrin-emodin, isouqueritin-gallic acid, catechin-emodin, catechin-gallic acid, and emodin-gallic acid), and the activities of these combinations against A. baumannii. The antibacterial activity was absent when standard compounds were administered alone and in binary combinations, while the activity was detected in the Rumex extracts by microdilution methods and bioautography assay. It suggests that interactions among these detected and other undetected compounds play a major role in the anti-A. baumannii activity. Further study should include higher concentrations of compounds (516 and 1 024 μg/mL) for a more precise estimation of their activity and interactions (additive or synergistic).

The bioautography showed that only fractions F6 and F8 did not express anti-A. baumannii activity and that active fractions/compounds are the most polar ones. The active fractions F1-F5 contained more polar compounds, flavonoid (quercetin-, kaempherol-, eriodictyol-) glycosides and/or anthraquinone (emodin-) glycosides that have not
been examined in the present study as a single agent, indicating their potential anti-\textit{A. baumannii} effect. In the inactive fractions, dominant compounds of F6 and F8 are gallic acid and catechin which had no considerable anti-\textit{A. baumannii} activity when they were tested alone. The activity of F7 suggests that either compounds eriodictyol and/or luteolin is potentially responsible for the antibacterial activity and/or rather all the compounds of the fraction F7 act synergistically. This assumption is supported by the previous report of eriodicyol antimicrobial activity with MICs ranging from 250-800 μg/mL against Gram-negative bacteria \textit{E. coli}, \textit{Salmonella enterica} subsp. \textit{enterica} serovar. Typhimurium, and \textit{Pseudomonas putida} and its synergistic activity in binary combination with hesperetin and naringenin\cite{39}. Similarly, luteolin was identified as a responsible component for antimicrobial activity of \textit{Rumex} extracts during food preservation\cite{40}. It is possible that, in general, synergism plays a major role in the extract activity when the MIC of the mixture was determined, and the individual compounds had weaker antimicrobial activity. Previously, it was reported that when all the active compounds based on the bioautography were isolated and characterized, they usually showed much lower activity than the expected, indicating the presence of the synergism\cite{41}. Besides the better activity of extracts than single compounds of binary combinations and potential additive/synergistic interactions of the components, the extracts have other beneficial properties, such as antioxidant\cite{42} and anti-inflammatory\cite{43}, which additionally can contribute to \textit{in vivo} anti-\textit{A. baumannii} effect. Thus, further experiments should focus on \textit{in vivo} study on the effect of topical application on the healing of wounds infected with \textit{A. baumannii}.

In this study, the antibacterial activity of the \textit{Rumex} extracts against MDR \textit{A. baumannii} isolates from wounds was confirmed, justifying their traditional application in the treatment of wound healing. Therapy options for wound infections due to MDR \textit{A. baumannii} are limited and both \textit{R. sanguineus} and \textit{R. crispus} extracts are potential natural alternatives. The agar-overlay HPTLC-bioautography coupled with LC/DAD/MS analysis of spotted fractions gave more insight into the types of secondary biomolecules contributing to the extract activity. The fractions of \textit{R. crispus} herb extract, containing flavonol (quercetin and kaempherol) glycosides, flavanon- (eriodictyol) and anthraquinone- (emodin) glycosides, and eriodictyol aglycone, have higher anti-\textit{A. baumannii} activity. This study supports further \textit{in vitro} and \textit{in vivo} studies on these ethnopharmacological remedies as a valuable and promising source of antibacterial compounds against MDR \textit{A. baumannii}.

**Conflict of interest statement**

We declare that there is no conflict of interest.

**Funding**

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant OI 172058.

**Authors’ contributions**

VAS, PK, and JN performed microbiological analyses, while ES, DO, and NMD performed chemical analyses. VAS and PK wrote the manuscript, and all authors discussed and analyzed the data. PK supervised the work.

**References**

[1] Almasaudi SB. \textit{Acinetobacter} spp. as nosocomial pathogens: Epidemiology and resistance features. \textit{Saudi J Biol Sci} 2018, 25(3): 586–596.

[2] Xie R, Zhang XD, Zhao Q, Peng B, Zheng J. Analysis of global prevalence of antibiotic resistance in \textit{Acinetobacter baumannii} infections disclosed a faster increase in OECD countries. \textit{Emerg Microbes Infect} 2018; 7(1): 31.

[3] da Silva KE, Maciel WG, Croda J, Cayô R, Ramos AC, de Sales RO, et al. A high mortality rate associated with multidrug-resistant \textit{Acinetobacter baumannii} ST79 and ST25 carrying OXA-23 in a Brazilian intensive care unit. \textit{PLoS One} 2018; 13(12): e0209367.

[4] Zhou H, Yao Y, Zhu BQ, Ren DH, Yang Q, Fu YQ, et al. Risk factors for acquisition and mortality of multidrug-resistant \textit{Acinetobacter baumannii} bacteremia: A retrospective study from a Chinese hospital. \textit{Medicine (Baltimore)} 2019; 98(13): e14937.

[5] Zarrilli R, Crispino M, Bagattini M, Barretta E, Di Popolo A, Triassi M, et al. Molecular epidemiology of sequential outbreaks of \textit{Acinetobacter baumannii} in an intensive care unit shows the emergence of carbapenem resistance. \textit{J Clin Microbiol} 2004; 4: 946-953.

[6] Seward RJ, Lambert T, Towner KJ. Molecular epidemiology of aminoglycoside resistance in \textit{Acinetobacter} spp. \textit{J Med Microbiol} 1998; 47: 455-462.

[7] Fournier PE, Vallenet D, Barbe V, Audic S, Ogata H, Poirel L, et al. Comparative genomics of multidrug resistance in \textit{Acinetobacter baumannii}. \textit{PLoS Genet} 2006; 2: e7.

[8] Isler B, Doi Y, Bonomo RA, Paterson DL. New treatment options against carbapenem-resistant \textit{Acinetobacter baumannii} infections. \textit{Antimicrob Agents Chemother} 2018; 63(1): e01110-e01118.

[9] Intorasoot A, Chornchoem P, Sookkhee S, Intorasoot S. Bactericidal activity of herbal volatile oil extracts against multidrug-resistant \textit{Acinetobacter baumannii}. \textit{J Interdiscil Ethnopharmacol} 2017; 6(2): 218–222.

[10] Tiwari V, Roy R, Tiwari M. Antimicrobial active herbal compounds against \textit{Acinetobacter baumannii} and other pathogens. \textit{Front Microbiol} 2015; 18(6): 618.

[11] Aleksic V, Mimica-Dukic N, Simin N, Nedeljkovic NS, Knezevic P.
Synergistic effect of *Myrtus communis* L. essential oils and conventional antibiotics against multi-drug resistant *Acinetobacter baumannii* wound isolates. *Phytotherapeutics* 2014; 21(12): 1666-1674.

[12] Newman DJ, Cragg GM. Natural products as source of new drugs over the last 25 years. *J Nat Prod* 2007; 70: 461-477.

[13] Vasas A, Orbán-Gyapai O, Hohmann J. The genus *Rumex*: Review of traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol* 2015; 175: 198-228.

[14] Denes A, Papp N, Babai D, Czúcz B, Molnár Z. Ehetö, vadon termő növények a Kárpát-medencében életű magyarak körében néprajzi és etnobotanikai kutatások alapján. In: Andrea D (ed.) termöőnövények és felhasználásuk a Kárpát-medencében élő magyarok 2015; 2007; 3: 35-76.

[15] Butura V. *Ehetö vadnövények a Kárpát-medencében* [in Romanian]. Bucharest, Romania: The Scientific and Encyclopedic Publishing; 1979.

[16] Baskan S, Daut-Özdemir A, Günaydin K, Erim FB. Analysis of anthraquinones in *Rumex crispus* by micellar electrokinetic chromatography. *Talanta* 2007; 71: 747-750.

[17] Shiwan S, Kumar Singh N, Hyeon Wang M. Carbohydrate inhibition and anti-cancerous and free radical scavenging properties along with DNA and protein protection ability of methanolic root extracts of *Rumex crispus*.* Nutr Res Pract* 2012; 6(5): 389-395.

[18] Pareek A, Kumar A. *Rumex crispus* L. –a plant of traditional value. *Drug Discovery* 2014; 4: 9: 20-23.

[19] Ahmed SS, Erum S, Khan SM, Nawaz M, Wahid A. Exploring the medicinal plants wealth: A traditional medico-botanical knowledge of local communities in Changa Manga Forest, Pakistan. *Middle-East. J Sci Res* 2014; 20: 1772-1779.

[20] Moerman D. *Native American ethnobotany*, Timber Press; 2003.

[21] Suh HJ, Lee KS, Kim SR, Shin MH, Park S, Park S. Determination of singlet oxygen quenching and protection of biological systems by various extracts from seed of *Rumex crispus* L. *J Phototach Photobiol B* 2010; 102(2): 102-107.

[22] Idris A, Wintola OA, Afolayan AJ. Phytochemical and antioxidant activities of *Rumex crispus* L. in treatment of gastrointestinal helminths in Eastern Cape Province, South Africa. *Asian Pac J Trop Biomed* 2017; 7(12): 1071-1078.

[23] Comara L, La Rocca A, Marsili S, Mariotti MG. Traditional uses of plants in the Eastern Riviera (Liguria, Italy). *J Ethnopharmacol* 2009; 125(1): 16–30.

[24] Clinical Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. 7th ed. Approved Standard M7-A7. CLSI, Wayne, PA; 2006.

[25] Knezovic P, Aleksić V, Simin N, Sivrić E, Petrović A, Mimica-Dukic N. Antimicrobial activity of *Eucalyptus camaldulensis* essential oils and their interactions with conventional antimicrobial agents against multi-drug resistant *Acinetobacter baumannii*. *J Ethnopharmacol* 2016; 175: 125-136.

[26] Orčić D, Francišković M, Bekvalac K, Svirčev E, Tkalec M, et al. Quantitative determination of plant phenolics in *Urtica dioica* extracts by high-performance liquid chromatography coupled with tandem mass spectrometric detection. *Food Chem* 2014; 143: 48–53.

[27] Yıldırım A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *J Agric Food Chem* 2001; 49(8): 4083-4089.

[28] Idris OA, Wintola OA, Afolayan AJ. Evaluation of the bioactivities of *Rumex crispus* L. leaves and root extracts using toxicity, antimicrobial, and antiparasitic assays. *Evid-Based Compl Alternative Med* 2019; 12: 682S-297.

[29] Gradishar H, Pirovskov P, Plaper A, Jeral A. Green tea catechins inhibit bacterial DNA gyrase by interaction with its ATP binding site. *J Med Chem* 2007; 50(2): 264–271.

[30] Mabe K, Yamada M, Ogumi I, Takahashi T. In vitro and in vivo activities of tea catechins against *Helicobacter pylori*. *Antimicrob Agents Chemother* 1999; 43(7): 1788-1791.

[31] Hamilton-Miller JMT. Chemical and biological properties of tea infusions. Frankfurt: U&M, Germany; 1997; p. 63-75.

[32] Yoda Y, Hu QZ, Zhao WH, Shumamura T. Different susceptibilities of *Staphylococcus* and Gram-negative rods to epigallocatechin gallate. *J Infect Chemother* 2004; 10(1): 55-58.

[33] Nitiema LW, Savadogo A, Simpson J, Dianou D, Traore AS. In vitro antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains. *Int J Microbiol Res* 2012; 3(3): 183-187.

[34] Razavi SM, Zahi S, Zarini G, Nazemiye H, Mohammadi S. Biological activity of quercetin-3-β-glucoside, a known plant flavonoid. *Bioorg Khim* 2009; 35(3): 376–378.

[35] Ajobeye TO, Skiebe E, Wilharm G. Phenolic acids potentiate colistin-mediated killing of *Acinetobacter baumannii* by inducing redox imbalance. *Biomed Pharmacother* 2018; 101: 737–744.

[36] Živković-Perica M, Hrenović J, Kugler N, Goić-Barisić I, Tkalec M. Antibacterial activity of *Pinus pinaster* bark extract and its components against multidrug-resistant clinical isolates of *Acinetobacter baumannii*. *Croatia Chemica Acta* 2015; 88(2): 133–137.

[37] Chukwujekwu JC, Coombes PH, Mulholland DA, van Staden J. Emodin, an antibacterial anthraquinone from the roots of *Cassia occidentalis*. *S Afr J Bot* 2006; 72(2): 295-297.

[38] Cooponsamy RM, Magwa ML. Antibacterial activity of aloe emodin and aloin A isolated from Aloe excelsa. *Afj J Biotech* 2006; 5(11): 1092-1094.

[39] Betts JW, Hornsey M, Wareham DW. In vitro activity of epigallocatechin gallate (EGCG) and quercetin alone and in combination versus clinical isolates of methicillin-resistant *Staphylococcus aureus*. *ASM 2014 Barts and the London, School of Medicine and Dentistry, UK; 2014.*

[40] Mahalla D, Bouaziz A, Ennouri K, Chawech R, Smaoui S, Jarraya B, et al. Antibacterial activity and bioguided fractionation of *Rumex tingitanus* extracts for meat preservation. *Meat Sci* 2017; 125: 22-26.

[41] Elliot JN, Katereere DR, McGaw LJ. The biological activity and chemistry of the southern African Combretaceae. *J Ethnopharmacol* 2008; 119: 689-699.

[42] Idris AO, Wintola OA, Afolayan AA. Phytochemical and antioxidant activities of *Rumex crispus* L. in treatment of gastrointestinal helminths in Eastern Cape Province, South Africa. *Asian Pac J Trop Biomed* 2017; 7(12): 1071-1078.

[43] Singh M, Purohit MC. Anti-inflammatory activity of methanolic extract of roots of *Rumex obtusifolius*. *Int J Pharm Sci & Res* 2018; 9(8): 3519-3522.