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J. Agric. Food Chem., Just Accepted Manuscript • DOI: 10.1021/acs.jafc.7b04202 • Publication Date (Web): 11 Nov 2017

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Research on chemical composition and biological properties including anti-quorum sensing activity of *Angelica pancicii* Vandas aerial parts and roots

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**ABSTRACT:** The essential oil, different extracts and isolated compounds of *Angelica pancicii* Vandas (Apiaceae) were investigated for the first time. The GC–FID and GC–MS analyses revealed sesquiterpenoids as the main constituents of *A. pancicii* essential oil of aerial parts with bornyl acetate (8.08%), *n*-octanol (5.82%), kessane (4.26%) and β-selinene (4.26%) as the main constituents. Analysis of methanol extracts, using HPLC–DAD/ESI–ToF–MS system, showed a total of 52 compounds in the aerial parts and 53 in the roots, indicated coumarins as the main constituents. In addition, new chromone (1) and six known furanocoumarins (2–7) were isolated from the roots and structurally elucidated by combined spectroscopic methods. The aerial parts extracts exhibited higher polyphenolic contents and antioxidant activity evaluated by three radical scavenging assays. Using micro–well dilution method, the strongest antibacterial activity profiles were determined for ethanol and methanol root extracts (minimum bactericidal concentrations (MBCs) = 0.25–3.00 mg/mL), which were comparable to the activity of streptomycin (MBCs = 0.34–1.24 mg/mL), while the strongest antibacterial compound of *A. pancicii* was oxypeucedanin hydrate (MBCs = 0.50–8.00 mg/mL). Antifungal potential was in moderate extent and the highest activity was obtained for roots methanol extract (minimum fungicidal concentrations (MFCs) = 4.00–14.00 mg/mL). Tested sub–minimum inhibitory concentrations (subMICs) of the extracts and isolated compounds inhibited selected *Pseudomonas aeruginosa* PAO1 virulence determinants. The most reduced growth of *P. aeruginosa* colony was in the presence of isolated oxypeucedanin. Ethanol (17.36–46.98%) and methanol (34.54–52.43%) roots extracts showed higher anti–biofilm activity compared to streptomycin (49.40–88.36%) and ampicillin (56.46–92.16%).

**KEYWORDS:** *Angelica pancicii*, chemical composition, coumarins, essential oil, antioxidant, antimicrobial, anti-quorum sensing activity
INTRODUCTION

The genus *Angelica* L. (Apiaceae) consists of about 50 species of tall, biennial and perennial herbs, native to temperate and subarctic regions of the Northern Hemisphere.\(^1\) *Angelica pancicii* Vandas is a perennial, endemic species of Balkan peninsula.\(^2\) The genus *Angelica* is well known for many nutritional and medicinal properties. More than half of *Angelica* species have pharmaceutical and ethno–medicinal utility and some of them are included in several national and European pharmacopoeias.\(^3\) Many angelicas are edible and a few are cultivated for food and alcohol industry usage. *Angelica archangelica* is traditionally used in the Far East and many parts of Western world. It was used by the native Sami population of northern Scandinavia for preservation of reindeer milk, preparation of cheese, as a natural sweetener and substitute for tobacco. Today, as World Economic Plant, it is grown commercially in many European countries for application of its aromatic root. The essential oil of the roots is well known as vanilla–like flavoring in commercial liqueurs, sweets and honey, while leaves are added to cooked fruit dishes, soups, fish or poultry.\(^4\) *A. sinensis* is also in culinary usage in China.\(^5\) In the US, this plant is marketed as a dietary supplement.\(^6\) Fresh leaves of herb *A. keiskei* are widely used for preparation of green juice and health–promoting food in Japan,\(^7\) while it is widely planted in Taiwan and consumed as a vegetable in many restaurants.\(^8\) *A. dahurica* is also frequently used as herbal ingredient in functional food and folk medicine,\(^9\) while highly valued aromatic plant of the Himalaya *A. glauca* is considered as useful cure for treatment of the gastrointestinal disorders.\(^10\) Many studies have confirmed various pharmaceutical properties of *Angelica* species, which are rich source of metabolites (essential oils, coumarins, acetylenic compounds,
chalcones, sesquiterpenes, polysaccharides, etc.) with broad spectrum of biological activities. Coumarins, the most characteristic chemical markers of the genus, could be considered as leading active principles of angelicas.\textsuperscript{11,12} As regards \textit{A. pancicii}, only essential oil of the plant was characterized so far, show in high content of monoterpenoids.\textsuperscript{13} From the genus \textit{Angelica}, only \textit{A. dahurica} (essential oil),\textsuperscript{14,15} and \textit{A. sinensis} (roots extracts)\textsuperscript{16} were tested for anti–quorum sensing (QS) activity on \textit{Pseudomonas aeruginosa} so far. A promising approach to combat this bacterium is to target its communication system by anti–QS agents through disruption of biofilm formation, flagella system, production of toxins, virulence enzymes \textit{etc}. The final result is pathogen more susceptible to the antibiotics. Some popular, edible species, e.g. culinary spice \textit{Curcuma longa}, were showed to attenuate the virulence of this bacillus on this way.\textsuperscript{17}

The subject of this study was the chemical characterisation of essential oil (EO) and methanol extracts (Es) obtained from \textit{A. pancicii}. Examination of EO chemical composition was performed by GC–MS/GC–FID system; methanol Es were analysed by HPLC–DAD–MS method and one new chromone (1) and six known furanocoumarins (2–7) were isolated from the root samples. Furthermore, since some \textit{Angelica} species are known as health promoting food, we determinated the biological activities of \textit{A. pancicii} Es and isolated compounds. Besides the quantification of phenolic and flavonoid contents, the screening of antioxidant, antimicrobial and anti–QS activities on \textit{P. aeruginosa} selected virulent factors of Es and isolated compounds was performed.

\textbf{MATERIALS AND METHODS}
**Chemicals and Apparatus.** Analytical grade solvents were provided from Zorka pharma (Šabac, Serbia) and before using for extraction and chromatographic separation were freshly distilled. For LC–MS and semi–preparative HPLC analyses, acetonitrile purchased from Merck KG (Darmstadt, Germany), formic acid (85% purity) from Lach–Ner, s.r.o. (Neratovice, Czech Republic) and Milli Q water 18.2 MΩ–cm, obtained from a Millipore Simplicity 185 purification system were used. For dry–column flash chromatography, silica gel 0.08 mm (Merck) was used. Analytical TLC was carried out on silica gel 60 GF254 20 × 20 cm plates, layer thickness 0.25 mm (Merck). Preparative HPLC was performed on an Agilent 1100 series instrument equipped with a DAD, using a Zorbax Eclipse XDBC–18 column (250 mm × 9.4 mm, 5 µm). Mass spectral (HR–ESI–MS) data were obtained from an Agilent Technologies 6210 time–of–flight LC/MS system. 1D and 2D NMR spectra were recorded on a Bruker Avance III 500 spectrometer (500.26 MHz for $^1$H and 125.80 MHz for $^{13}$C nuclei) equipped with 5mm broadband probehead (BBO). The spectra were measured at room temperature (298K) in CDCl$_3$ for all compounds, with addition of 4 drops of MeOD only for compound 1 in order to increase its solubility. All spectra were referenced to the residual solvent’s signal (7.26 ppm and 77 ppm for $^1$H and $^{13}$C respectively). For 2D spectra, H–H COSY, H–H NOESY, H–C HSQC and H–C HMBC, the standard Bruker pulse sequences were applied (cosygpmfqf, noesygpph, hsqetgpsi2, hmbcgplpndqf). IR spectra were recorded on a ThermoScientific Nicolet 6700 FT–IR spectrometer using a capillary film technique. Optical rotations were measured on a Rudolph Research Analytical AUTOPOL IV automatic polarimeter. Elemental analysis was performed on a Vario EL III C,H,N,S/O elemental analyzer (Elementar). The spectrophotometric measurements were performed using JENWAY 6306 UV/Vis spectrophotometer.
Gallic acid (GA) (98%), 3–tert–butyl–4–hydroxyanisole (BHA), 2,2–dyphenyl–1–picrylhydrazyl (DPPH), Folin–Ciocalteu phenol reagent, potassium acetate (≥99%), aluminum trinitrate nonahydrate (≥98%), dimethyl sulfoxide dried and β–carotene (analytical grade) and p–iodonitrotetrazolium violet color (INT) were obtained from Sigma–Aldrich Co., St Louis, MQ, USA. Sodium carbonate anhydrous (analytical grade) was obtained from Centrohem doo (Stara Pazova, Serbia). Potassium peroxidisulphate (≥99%), L(+–)–ascorbic acid, Tween 80 and linoleic acid (analytical grade) were obtained from Acros organics, Fisher Scientific UK Ltd., Loughborough, Leicestershire, UK. 2,2′–Azino–bis(3–ethylbenzothiazoline–6–sulphonic acid(ABTS) and quercetin hydrate (QE) (≥98%) were purchased from TCI Europe NV, Boerenveldsweg, Belgium. Mueller–Hinton Agar (MH), Malt Agar (MA) and Tryptic Soy Broth (TSB) were obtained from the Institute of Immunology and Virology, Torlak (Belgrade, Serbia), while streptomycin and ampicillin solutions (100 µg/mL) from Hyclone (Logan, Utah, USA). Dimethylsulfoxide (DMSO, ≥99.9%) was purchased from Merck KGaA, Germany. Antimicotic Diflucan (containing 50 mg fluconazole) was obtained from Pfizer PGM, Pocesur–Cisse, France.

**Plant Material.** Plant material in pre–flowering stage was collected at Mt. Pelister, a national park which is in the surrounding of Bitola city in Republic of Macedonia (GPS: N 41°01′58″; E 21°11′59″) in July, 2013 and 2014. It was determined as *Angelica pancicii* Vandas by Prof. V. S. Matevski. Voucher specimens of *A. pancicii* (BU16776 (2013) and BU16672 (2014)) are deposited at the Herbarium of the Institute of Botany and Botanical Garden “Jevremovac”, (BEOU), Faculty of Biology, University of Belgrade, Serbia.

**EO Isolation.** Greenish EO of *A. pancicii* was isolated from dry plant material (200 g) by hydrodistillation (3h) using Clevenger type apparatus. Obtained oil (nonquantifiable yield) was refrigerated in a sealed vial prior to the chemical analysis.
Extraction Procedure 1. Methanol (ME), ethanol (EE) and aqueous extracts (AE) of A. *pancicii* aerial parts and roots collected in 2013 were obtained by the ultrasonic (US) extraction conducted in dark conditions. The procedure for US extraction was followed according to modified method described by Džamić et al.\textsuperscript{19} 10g of each grounded sample were extracted for 24 h with 200 mL of listed solvents using an ultrasonic apparatus for the first and the last hour of extraction. Afterwards, the filtration was carried out using Whatman filter paper No1. MEs and EE2 were evaporated under reduced pressure at maximum temperature of 40 °C, while frozen AEs were lyophilized. Upon measurements (Table 1), obtained Es were packed in glass bottles and kept under refrigeration until further utilization.

Extraction Procedure 2. The air–dried *A. pancicii* roots (50g), collected in 2014, were powdered and extracted with hexane (650 mL) in a Soxhlet apparatus (3 h) and re–extracted with dichloromethane (DCM, 600 mL), yielding 2.0032g of hexane and 1.0746g of DCM extract.\textsuperscript{20,21}

GC Analysis with FID and MS Detection. The analysis of the oil was carried out on a GC HP–5890 II apparatus, equipped with split–splitless injector, attached to HP–5 column (25 m × 0.32 mm, 0.52 \(\mu\)m film thickness, Agilent Technologies, Waldbronn, Germany) and fitted to FID. Carrier gas flow rate (H\(_2\)) was 1 mL/min, split ratio 1:30, injector temperature was 250 °C, detector temperature 300 °C; column temperature was linearly programmed from 40–240 °C (at rate of 4 °/min). The same conditions were employed for Gas Chromatography–Mass Spectrometry (GC–MS) analysis; HP G 1800C Series II GCD system equipped with HP–5MS column (30 m × 0.25 mm, 0.25 \(\mu\)m film thickness, Agilent Technologies, Waldbronn, Germany) was used. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV) in \(m/z\) range 40–400. Identification of EO components was accomplished by matching mass spectral data with those held in Wiley 275 mass spectral library and comparison of obtained
retention indexes with Adams 0.4. RI library data\textsuperscript{22} using AMDIS software. For the purpose of relative abundance determination, percentages of peak area of compounds relative to the total area obtained by Flame Ionization Detector (FID) were used.

**LC Analysis with DAD and MS Detection.** The analysis of the MEs of the roots and the aerial parts obtained by *Extraction procedure 1* were performed using HPLC–DAD/ESI–ToF–MS system consisting of an HPLC instrument Agilent 1200 Series (Agilent Technologies, Waldbronn, Germany) equipped with a degasser, a binary pump, an auto–sampler, a termostated column compartment and a diode array detector (DAD) and coupled with a 6210 Time–of–Flight LC/MS system (Agilent Technologies, Santa Clara, California, USA) via an electro spray ionization (ESI) interface. Immediately before analysis, the dry residues of MEs were re–dissolved in 1 mL acetonitrile–methanol (95:5) and samples (c=10.0 mg/mL) were filtered through Captiva Premium Syringe Filter Agilent Technologies (0.45 µm × 25 mm) and, in a volume of 5 µL, injected into a Zorbax Eclipse Plus C18 (150 mm × 4.6 mm i. d.; 1.8 µm) column, maintained at 40 °C. The mobile phase was a mixture of solvent A (0.20% formic acid in water) and solvent B (acetonitrile) according to a combination of isocratic and gradient modes of elution: 0–1.5 min, 95% A, 1.5–26 min, 95–5% A, 26–35 min, 5% A, 36–41 min, 95% A, at a flow rate of 1.40 mL/min. Detection was accomplished using DA detector and storing the signals in the wavelength range from 190–650 nm. The HPLC effluent was directed into the atmospheric pressure ESI ion source of the mass spectrometer. The eluted compounds were mixed with nitrogen in the heated nebulizer interface and the polarity was tuned to positive/negative. An adequate calibration of the ESI parameters (capillary voltage, gas temperature, nebuliser pressure, and fragmentor voltage) was required to optimise the response and to obtain a high sensitivity of the molecular ion. The MS conditions were as follows:
capillary voltage, 4000 V; gas temperature, 350 °C; drying gas, 12 mL/min; nebuliser pressure, 45 psig; fragmentor voltage, 140 V; mass range, 100–2000 m/z. A personal computer system running Mass Hunter Workstation software was used for data acquisition and processing. The Molecular Feature Extractor of Mass Hunter Workstation was used to predict chemical formulas.

**Isolation Procedure 1.** A part of roots ME (1.5g) obtained by Extraction procedure 1 was dissolved in destilated H₂O and re–extracted with DCM (3 × 100 mL), ethyl–acetate (3 × 100 mL) and butanol, consecutively. Organic phases were drying with anhydrous Na₂SO₄, filtrated, evaporated and analysed by TLC, LC/MS and NMR. DCM fraction (649.2 mg) was chosen for further separation by silica gel dry–flash column chromatography (80g, 18 × 2.2 cm²). The elution started with petrol (4.9 mg) and continued as follows: Et₂O (fraction F₂, 260.4 mg), Et₂O–DCM 1:1 (F₃, 127.9 mg), DCM (10.7 mg), DCM–MeOH 99:1 (1.5 mg), 95:5 (1.1 mg), 9:1 (F₇, 95.3 mg), 85:15 (23.2 mg), 8:2 (18.7 mg), 75:25 (10.0 mg), 6:4 (7.6 mg), 4:6 (9.1 mg), methanol (5.8 mg). Each fraction was eluted with 50 mL of the solvent system. Fractions F₂, F₃ and F₇ were further fractionated by semi–preparative HPLC–DAD to isolate pure compounds using a 0.02% HCOOH/ACN (acetonitrile) elution system with a flow rate of 4 mL/min and the following gradient program: 0–2 min, 50% ACN; 2–17 min, 50–65% ACN; 17–20 min, 65% ACN. The detection wavelengths were 220, 260, 280, 320 and 360 nm. A part of fraction F₂ (200 mg) was divided into two fractions, yielding coumarin saxalin (4, Rₜ 9.87–10.49 min, 2.583 mg) and a mixture of oxypeucedanin and oxypeucedanin hydrate (3 + 6, Rₜ 9.14–9.77 min). Due to the fact that 0.02% HCOOH was used for fractionation, it was assumed that dihydroxide 6 was formed from epoxyde 3 by ring–opening. Thus, a rest of F₂ was purified without HCOOH, with H₂O/ACN elution system and the same gradient program. Oxypeucedanin (3, 4.647 mg) was isolated at the same retention time. Applying a semi–preparative HPLC–DAD under the former
conditions, coumarins t–OMe–oxypeucedanin hydrate (2, R<sub>t</sub> 7.2–7.7 min, 7.442 mg), saxalin (4, R<sub>t</sub> 10.1–10.5 min, 3.216 mg), ostruthol (5, R<sub>t</sub> 11.9–12.4 min, 22.591 mg) and the new chromone 5′–acetylenidimol A (1, R<sub>t</sub> 8.6–9.2 min, 6.8 mg) were isolated from F3, as well as oxypeucedanin hydrate (6, 12.959 mg) from F7. The structures of isolated compounds are given in Figure 1.

**Isolation Procedure 2.** DCM extract obtained by Soxhlet extraction was further separated by semi–preparative HPLC using a H<sub>2</sub>O/ACN elution system with a flow rate of 4 mL/min and the following gradient program: 0–2 min, 50% ACN; 2–17 min, 50–65% ACN; 17–20 min, 65% ACN. Besides previously isolated compounds, 1 (R<sub>t</sub> 8.43–9.09, 11.876 mg), 3 (R<sub>t</sub> 9.17–9.77, 10.507 mg), 4 (R<sub>t</sub> 9.88–10.4 min, 6.219 mg) and 5 (R<sub>t</sub> 11.66–12.43 min, 31.076 mg), isoimperatorin was also isolated (7, R<sub>t</sub> 17.18–17.73 min, 3.180 mg).

**Determination of Total Phenolic and Flavonoid Contents and Antioxidant Activity.** Measurements of total phenolic and flavonoid contents (TPCs and TFCs) of tested samples were performed. Additionally, evaluation of free radical scavenging activity of Es of aerial parts and roots obtained by Extraction procedure 1 was conducted by DPPH, ABTS and β–carotene bleaching (BCB) tests. All experiments were carried out by well–known and commonly used methods<sup>24–28</sup> (described in the Supporting Information S1).

**Antimicrobial Activity. Preparation of Stock Solutions of Es and Isolated Compounds.** Crude A. pancicii Es obtained by Extraction Procedure 1 were dissolved in 5% DMSO to obtain stock solutions (20 mg/mL). Purified compounds were dissolved in 5% DMSO in addition of Tween 40 (in concentration of 0.1%) to gain stock solution of 2 mg/mL. Subsequently, different dilutions of these solutions were examined against 8 bacteria, 7 fungi and 1 yeast to determinate their antimicrobial potency.
Microbial Cultures Treated Isolates. For testing of investigated samples, pure control strains were obtained from mycological laboratory, Department of Plant Physiology, Institute for Biological Research “Siniša Stanković”, Belgrade, Serbia. The following bacteria were used: *Bacillus cereus* (food isolate), *Micrococcus flavus* (ATCC 10240), *Listeria monocytogenes* (NCTC 7973) and *Staphylococcus aureus* (ATCC 6538) of Gram positive and *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35210), *Enterobacter cloacae* (human isolate) and *Salmonella typhimurium* (ATCC 13311) of Gram negative bacteria. Listed fungi were used: *Trichoderma viride* (IAM 5061), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 10509), *Aspergillus fumigatus* (ATCC 9197), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger* (ATCC6275) and yeast *Candida albicans* (ATCC 10231). Dilutions of bacterial inocula were cultured on solid MH medium, while fungi were maintained on solid MA medium. The cultures were subcultured once a month and stored at +4 °C for further usage.

Micro–Well Dilution Assay. The antimicrobial activity of *A. pancicii* samples was assayed by modified microdilution method described by CLSI and Hanel and Raether. The technique was carried out in sterile 96–well microtiter plates, by adding different 5% DMSO dilutions of Es and isolated compounds into corresponding medium – TSB and MA, for bacteria and fungi, respectively. Prior to experiment, bacterial and fungal inocula were prepared. For bacterial strains, 100 µL of overnight cultures were mixed with 900 µL of medium to obtain the concentration of 1.0 × 10^8 colony forming units (CFU)/mL. Fungal inocula were prepared by washing spores with sterile 0.85% saline solution (which contains 0.1% Tween 80 (v/v)). The microbial cell suspensions were adjusted with sterile saline to a concentration of approximately 1.0 × 10^6 CFU/mL for bacteria and 1.0 × 10^5 CFU/mL for fungi in a final volume of 100 µL per
well. Incubation of bacteria at 37 °C lasted for 24 h, and for fungi 72 h at 28 °C. The minimum inhibitory concentrations (MICs) are defined as the lowest concentrations of tested samples, completely inhibiting the growth of used pathogens. The lowest concentrations with no visible growth after serial sub-cultivation, indicating 99.5% killing of the original inoculums, are determined as the minimum bactericidal/fungicidal concentrations (MBCs/MFCs). The growth of tested bacteria was determined by a colorimetric microbial viability assay, based on reduction of a 0.2% INT aqueous solution and compared with positive control for each strain. Two repeats were done for each sample. The 5% DMSO streptomycin solution (1 mg/mL) was used as positive control for bacteria. The solution of standard fluconazole (2 mg/mL) was included for fungi. Sterilized distilled water containing 0.02% Tween 80 and 5% DMSO was used as negative control.

**Anti–QS Activity. Bacterial Strains, Growth Media and Culture Conditions.** For performing the experiments, *Pseudomonas aeruginosa* PAO1 from the collection of the Mycoteca, Institute for Biological Research "Siniša Stanković", Belgrade (Serbia), was used. The strain was routinely grown in Luria–Bertani (LB) medium (1% w/v NaCl, 1% w/v Tryptone, 0.5% w/v yeast extract) with shaking (220 rpm) and cultured at 37°C.

**Biofilm Formation.** To determinate the effect of *A. pancicii* Es and isolated compounds on *P. aeruginosa* biofilm formation, the method described by Drenkard and Ausubel and Spoering and Lewis with some modifications was followed. The anti–biofilm forming ability of different concentrations (0.125, 0.25 and 0.5 of MICs) of samples was tested, using polystyrene flat–bottomed microtitre 96–well plates. Subsequently after pipetting 100 µL of sub–inhibitory concentrations (subMICs) of tested samples and 100 mL of medium (control), 100 µL of *P. aeruginosa* overnight culture inoculum was added into each well of the plate.
incubation at 37 °C lasted for 24 h and after that each well was washed twice with sterile phosphate buffered saline (PBS, pH 7.4) and dried. In order to determine the biofilm mass, 0.1% crystal violet was poured and left for 10 min. After drying, 200 µL of 95% ethanol (v/v) was added to solubilise the dye that had stained the biofilm cells. The excess stain was washed off with distilled H₂O. After 10 min, the content of the wells was homogenized and the absorbance at λ = 625 nm was read on a Sunrise™ –Tecan ELISA reader. The experiment was done in triplicate and repeated two times. The values were presented as a mean values ± standard deviation (SD).

Twitching and Flagella Motility. The cells of P. aeruginosa, grown in the presence or the absence of Es and isolated compounds, were washed twice with sterile PBS and re–suspended in PBS at 1 × 10⁸ CFU/mL (optical density (OD) of 0.1 at 660 nm). In brief, the cells were stabbed into a nutrient agar plate with a sterile tooth pick and incubated overnight at 37 °C. Plates were then removed from the incubator and incubated at room temperature for 48 h. Colony edges and the zone of motility were measured with a light microscope.³⁵,³⁶ 0.5MICs of samples were mixed into 10 mL of molten MH medium and poured immediately over the surface of a solidified LB agar plate as an overlay. The plate was point inoculated with an overnight culture of PAO1 once the overlaid agar had solidified and incubated at 37 °C for 3 days. The extent of swimming was determined by measuring the area of the colony.³⁷ The experiment was done in triplicate and repeated two times. The colony diameters were measured three times in different direction and values were presented as a mean values ± SD.

Pyocyanin Production. The quantification of ability of tested Es and isolated compounds to inhibit P. aeruginosa pyocyanin production was conducted using the flask assay. P. aeruginosa overnight culture was diluted to OD₆₀₀ nm 0.2. After adding the samples, dissolved
in 5% of DMSO (0.5MICs = 0.50–5.00 mg/mL for Es and 0.25–8.00 mg/mL for compounds) to 5mL of bacteria inoculum dilution, the 24 h incubation at 37 °C ensued. Thereafter, the treated cultures were extracted with chloroform (3 mL), followed by mixing the chloroform layer with 0.2 M HCl (1 mL). The absorbance readings (520 nm) of the extracted organic layer were performed using a Shimadzu UV1601 spectrophotometer (Kyoto, Japan). The experiment was done in triplicate and repeated two times. The values were expressed as ratio (OD_{520}/OD_{600})\times100.

Statistical Analysis. For tested Es and compounds, three samples were used and all assays were carried out in triplicate. The results are expressed as mean values and standard deviation. The results were analyzed using one–way analysis of variance (ANOVA) followed by Tukey’s HSD Test with a = 0.05. This analysis was carried out using SPSS v. 18.0 program.

RESULTS AND DISCUSSION

Chemical Analysis of *A. pancicii*. Chemical Composition of EO. According to obtained results from GC–FID/GC–MS analyses, 81 compound was identified in *A. pancicii* EO (Table 2). Tested oil had the highest percentage of oxygenated sesquiterpenes (34.96%), followed by sesquiterpene hydrocarbons (21.88%) and oxygenated monoterpenes (19.22%). Also, fatty acid derivatives (12.36%), two oxygenated diterpenes (4.41%) and one diterpene hydrocarbon (3.87%) were determined. The alcohols were predominant among oxygenated monoterpenes, sesquiterpenes and fatty acid derivatives (8.88%, 21.32%, 7.91%, respectively). The ester bornyl acetate was the main constituent of *A. pancicii* oil (8.08%) followed by fatty alcohol *n*–octanol (5.82%) and sesquiterpenoids kessane (4.26%) and \(\beta\)–selinene (4.26%). Other compounds were
present in smaller amounts (<4%) (Table 2). According to literature data, monoterpenic hydrocarbons are the most abundant in EOs of many species of this taxa.\textsuperscript{38–40} Also, a significant fraction of phthalides can be found in EO of some representatives.\textsuperscript{41,42} In this study, the analysis of \textit{A. pancicii} EO showed the highest percentage of sesquiterpenoids (56.84%). In contrast, using GC, GC–MS and HS–GC–MS techniques, Simonović et al.\textsuperscript{13} showed that EO obtained from \textit{A. pancicii} aerial parts originated from mt. Vidlič (Serbia) possesses high content of monoterpenoids (92.8% and 97.7%, respectively) and notably lower percentage of sesquiterpenoids (4.5% and 1.2%, respectively). As the main constituents they identified β–phellandrene, α–pinene and α–phellandrene, while bornil acetate, which was the most abundant in our study, was recorded in lower percentage (1.3% (GC, GC–MS) and 0.8% (HS–GC–MS)). Also, δ–3–karene and mircene were not recorded in the sample from mt. Pelister, while these compounds were present in significant amounts in EO from Vidlič (GC, GC–MS).\textsuperscript{13}

\textit{Tentative Analysis of MEs.} In order to preliminary identify secondary metabolites of \textit{A. pancicii}, MEs of the aerial parts and roots were analysed on a HPLC–DAD/ESI–TOF–MS, in ESI\textsuperscript{+} and ESI\textsuperscript{−} modes. The compounds were structurally assigned in accordance with the exact molecular masses/formulas (Tables 3 and 4). UV data also provided evidence for structure confirmation. The most abundant components of this plant are coumarins, which are characteristic of the genus and could be found in all plant parts.\textsuperscript{12}

A total of 52 compounds were identified in ME of aerial parts and 53 in ME of the roots (Tables 3 and 4).\textsuperscript{13,43–84} Specified structures are mostly in line with literature on \textit{Angelica} species and/or Apiaceae family. Coumarins are represented with 32 compounds found in each analysed plant part: simple coumarins, coumarin glucoside isoscopoletin β–D–glucopyranoside, furanocoumarins (including furanocoumarin ethers of falcarindiol–japoangelols C and D and...
furanocoumarin glycoside nodakenin), pyranocoumarins, and bifuranocoumarins dahuribin D and rivulobirin A. Besides, this analysis revealed a presence of benzofuran derivatives (e.g. glycosidecnidioside A), chromones (e.g. noreugeninan 3′–O–angeloylhamaudol), chalcones (xanthoangelol J, C and E), sesquiterpenes, polyacetylenes (falarinone and falcarnindiol), fatty acid (pinelic acid) and fatty alcohol (9,12,15–octadecatrien–1–ol), flavonoid glucoside hirsutrin, ester glyceryl linoleate, 3–caffeoyl quinic acid, glycosylated hydroquinone arbutin and lignin kaerophylin (Tables 3 and 4). All identified coumarins have been found previously in the Angelica species except bifuranocoumarin rivulobirin A, which was isolated from the roots of Heracleum rapula (Apiaceae). Kaerophylin, 3′–O–angeloylhamaudol, falarinone, linolenic and pinelic acid have not been found in the genus Angelica so far, but they were found in some species of Apiaceae family. According to literature data, only few angelicas contained chromones e.g. A. archangelica, A. japonica and A. polymorpha.

Isolation and Structure Elucidation. One new chromone (1) and five furanocoumarins (2–6) were isolated from the crude ME of the roots re–extracted with DCM (plant material collected in 2013). The roots collected in 2014, after a Soxhlet extraction and further separation by semi–preparative reversed phase HPLC, gave furanocoumarin 7, together with compounds 1, 3–5. According to their NMR, mass spectra, [α]$_D^{22}$ (Supporting Information S2), and a comparison with previously reported spectroscopic data, isolated furanocoumarins were identified as follows: t–OMe–oxyypeucedanin hydrate (2), oxyypeucedanin (3)$^{53}$ (Supporting Information S13–S20), saxalin (4), ostruthol (5)$^{67}$ oxyypeucedanin hydrate (6) and isoimperatorin (7)$.^{48}$ All of them are linear furanocoumarins with C–5 substitution (Figure 1), derived from isoimperatorin (5–isopentenyloxypsoralene).
The new compound 1 was determined as 5’–acetylcnidimol A on the bases of IR, HR–ESI–MS, 1D and 2D NMR spectra (Supporting Information S3–S12) and a comparison with literature which showed that new chromone is derivative of cnidimol A previously found in Cnidium japonicum.\textsuperscript{85} Cnidimol A is bioactive metabolite which showed high anti-inflammatory activity by significant inhibition of formyl-l-methionyl-l-leucyl-l-phenylalanine/cytochalasin B-induced $\text{O}_2^*$ generation and/or elastase release.\textsuperscript{86} Related compound cnidimoside B is anti-adipogenic chromone which effectively inhibited adipocyte differentiation.\textsuperscript{87}

The compound 1 was isolated as a white powder. Molecular formula C\textsubscript{17}H\textsubscript{18}O\textsubscript{6} was deduced from the positive ion mode HR–ESI–MS ($m/z$ 319.1184 [M+H]\textsuperscript{+}; calcd. 319.1176 and $m/z$ 341.0987 [M+Na]\textsuperscript{+}; calcd. 341.0996). The IR spectrum showed absorption bands characteristic of a hydroxyl (3342 cm\textsuperscript{−1}), an acetyl (1719 cm\textsuperscript{−1}), a pyron carbonyl (1639 cm\textsuperscript{−1}) and an aromatic ring (1439 cm\textsuperscript{−1}). In the $^1$H NMR spectrum (Table 5), a broad singlet of the methyl group at C–2 (CH\textsubscript{3}–11, $\delta$ 2.15 s), which is coupled with olefinic proton of $\gamma$–pyrone ring H–3 ($\delta$ 5.81 br s), together with singlet at $\delta$ 6.16 originated from the aromatic proton H–8, indicated the basic chromone structure of compound 1, and a 2,5,6,7–substitution. COSY signal CH\textsubscript{3}–11/H–3 confirmed this interpretation. Remaining $^1$H NMR signals belonged to the substituent at C–6, while signals from OH–5 and OH–7 were missing. 2–Methyl–2–butenyl acetate at C–6 position was deduced from signals of methyl groups OCOCH\textsubscript{3} and CH\textsubscript{3}–4’, an olefinic proton H–2’ and methylene protons H\textsubscript{2}–1’ and H\textsubscript{2}–5’ (Table 5). The following coupling patterns in the COSY spectrum enabled identification of these protons: CH\textsubscript{3}–4’/H–2’, H\textsubscript{2}–1’, H–2’/H\textsubscript{2}–1’. Carbons were assigned from $^{13}$C, DEPT, HSQC and HMBC spectra. DEPT spectrum pointed out two secondary carbons at $\delta$ 20.7 and 63.4, which correlations in HSQC spectrum
revealed C–1’ and C–5’, respectively. Combining of DEPT and HSQC spectra revealed also methyl groups CH$_{3}$–11, OCOCH$_{3}$ and methine carbons C–8, C–3 and C–2’. The lowest field signals at $\delta$ 182.3 and 171.9 originated from carbonyls, which were identified as C–4 and OCOCH$_{3}$, respectively, on the basis of HMBC correlations C–4/H–3 and H$_{2}$–5’, OCOCH$_{3}$/OCOCH$_{3}$. The C–6 iso–butenyl substituent position was confirmed by correlations H$_{2}$–1’/C–5, C–6 and C–7. The rest of carbons were mainly assigned by means of HMBC correlations which are given in Table 5. NOESY correlations (Table 5) H$_{2}$–1’/H–5’ and CH$_{3}$–4’/H–2’, H–5’ provided evidence for the Z–configuration of double bond. Elemental analysis (found C – 64.00, H – 5.94; requires: C – 64.14, H – 5.70%) confirmed the structure and purity of the compound.

**Analyses of Biological Activities of A. pancicii. TPCs, TFCs and Antioxidant Activity of Es.** Obtained results showed different phenolic contents and varying degrees of antiradical activity of A. pancicii Es (Table 6). In general, Es of aerial parts had higher phenolic (TPCs = 72.77–143.99 mg GA/g of DE) and flavonoid contents (TFCs = 4.00–35.15 mg QE/g of DE) in comparison to the Es of roots. The highest TPC possessed EE of aerial parts, while ME followed by EE of aerial parts was richest in TFCs. According to presented results (Table 6), EE of aerial parts exhibited the strongest antioxidant activity, which was in accordance with the highest TPC. All together, the aerial parts Es exhibited stronger scavenging activity in comparison to the roots Es in DPPH (IC$_{50}$ = 0.26–0.29 mg/mL for aerial parts; IC$_{50}$ = 0.40–0.47mg/mL for roots) and BCB tests (IC$_{50}$ = 2.45–2.98 mg/mL for aerial parts; IC$_{50}$ = 4.94–14.00 mg/mL for roots), but lower comparing to controls BHA and vit. C (IC$_{50}$ = 0.03–1.22 mg/mL). AE of roots exhibited the lowest antioxidant potential which coincides with the results obtained for TPC (Table 6).
Literature data showed that EOs and Es of various plant parts of *Angelica* species possessed antioxidant activity in a concentration–dependent manner, which is in accordance with results obtained in this work. Similarly to *A. pancicii* Es, *A. koreana* EO and its main components showed significant dose–dependent scavenging activity in DPPH test. For coumarins oxypeucedanin and oxypeucedanin hydrate which were isolated from *A. pancicii* in this study, previously was proven to possess modest antioxidant and cytotoxic activity.

**Antibacterial Activity of Es and Isolated Compounds.** The results obtained for *A. pancicii* antibacterial activity (Table 7) revealed that tested samples expressed strong to moderate inhibitory effect on used bacteria. Es obtained from the roots showed the strongest activity among all tested samples, while isolated compounds manifested the minimum inhibition capacity. According to obtained results, EE and ME of the roots had MBCs values in range with those of streptomycin (MBCs = 0.25–5.00 mg/mL). Oxypeucedanin hydrate was the strongest antibacterial agent among compounds, killing all bacteria in the range of MBCs = 0.50–8.00 mg/mL, followed by tert–O–methyl oxypeucedanin hydrate. The most sensitive bacteria were *B. cereus* and *S. aureus*, while the most resistant strains were *L. monocytogenes*, *P. aeruginosa* (Es), *E. coli* and *E. cloacae* (compounds). ME of *A. pancicii* aerial parts showed moderate activity in our research and given results are comparable to previously examined ME of *A. lucida* fruits and its constituents isoimperatorin and oxypeucedanin hydrate, which were also found in ME of *A. pancicii*. Besides, Gram positive and negative bacteria displayed similar sensitivity to both – *A. pancicii* and *A. lucida* species and *P. aeruginosa* appeared to be one of the most resistant strains in both studies.

**Antifungal Activity of Es.** Tested fungi showed higher resistance to investigated samples compared to the bacteria. Obtained results for antifungal activity (Table 8) indicated moderate to
low potential of Es. EE of roots had the strongest antifungal effect (MFC = 8.00–12.00 mg/mL).

*P. funiculorum* was the most sensitive fungus (MFCs = 8.00–18.00 mg/mL), followed by *C. albicans* and *T. viride*, while the most resistant were *A. ochraceus* and *A. niger* (MFCs = 12.00–18.00 mg/mL for both strains). *A. pancicii* samples expressed modest activity against *C. albicans*, while Es and isolated compounds of *A. lucida* were inactive against assayed *Candida* species. The *Aspergillus* fungi were less sensitive to *A. pancicii*Es and this is in agreement with the observations of Roh and Shin, who found that this strain was less vulnerable to activity of *A. koreana* EO. Coumarins oxypeucedanin and oxypeucedanin hydrate, which were detected in *A. pancicii* MEs, manifested good antimicrobial activity in previous study of Stavri and Gibbons and Razavi and Zarrini.

**Anti–QS Activity of Es and Isolated Compounds.** All tested samples showed inhibitory effect against *P. aeruginosa* with MICs values in the range of 1.00–5.00 mg/mL for Es and 1.00–16 mg/mL for compounds, which led us to further anti–QS examination of the samples on selected *P. aeruginosa* PAO1 determinants. According to the results given in Table 9, all tested substances were effective in the presence of 0.5MIC. Considering all tested amounts, Es were more effective in comparison to isolated metabolites. In the presence of Es biofilm synthesis of *P. aeruginosa* occurred in lower range (17.36% – 74.53%) than in the presence of ampicillin and streptomycin (49.40% – 92.16%). The highest inhibition activity was observed for EE of the roots (inhibition of 82.64%). Isolated coumarins and new chromone were active in the range of 2.72% (0.125MIC of oxipeucedanin hydrate) to 71.60% (0.5MIC of isoimperatorin). The most promising anti–biofilm agents among compounds were osthrutol and oxypeucedanin hydrate.

The observation of *P. aeruginosa* twitching and flagella motility and colony formation indicated white to green coloration and modified diameters of treated colonies (Table 9). The
most of the colony edges had regular or tiny flagella with size from 16 to 160 µm except colony with acetyl cnidimol A (280 µm). The maximum reduction in diameter was observed in the presence of oxypeucedanin (8.66 mm), followed by EE of roots (9.33 mm) and AE of roots (11.00 mm), and no flagella were noticed. Reduced protrusions were noticed after application of oxypeucedanin, oxypeucedanin hydrate and all Es except AE of aerial parts (Figure 2).

The production of pyocyanin was reduced by all A. pancicii samples. Tested Es (0.5MIC) demonstrated inhibitory activity against the production of this green pigment (77.49% – 114.59%) when compared to the control P. aeruginosa (141.55%) (Figure 3). EE of aerial parts showed better inhibition in comparison with streptomycin (84.27%), while AE of roots exhibited better anti–pyocyanin effect (84.68%) than ampicillin (97.56%). The most effective anti–pyocyanin agent among isolated compounds was tert–O–methyl oxypeucedanin hydrate, enabling 91.74% of pyocyanin production in contrast to saxalin which allowed 133.54% of its synthesis. New chromone, acetyl cnidimol A, interfered pyocyanin production in similar scale as ampicillin (93.72% and 97.56%, respectively) (Figure 3).

Earlier reports revealed anti–QS activity of some species from Angelica genus. Previous results obtained for anti–QS activity of A. dahurica roots ME pointed out this sample as one of the most effective among 97 tested methanol plant Es against P. aeruginosa PAO1.15 Chong et al.14 confirmed that Es of A. dahurica roots exhibited anti–QS properties on P. aeruginosa. Also, A. sinensis exhibited anti–QS activity by inhibiting selected virulence determinants of the P. aeruginosa PAO1.16 Considering all results of anti–QS tests in this work, Es of the roots had moderate potential against QS of PAO1, similar to acetone–aqueous extract of A. sinensis roots.16 MEs of A. pancicii with coumarins as the main constituents, exhibited strong anti–biofilm activity. It was proven that furanocoumarins hinder the formation of biofilm in P.
aeruginosa, E. coli and S. typhimurium\textsuperscript{93}, indicating the main role of this compounds in strong anti–biofilm activity of A. pancicii MEs.

In conclusion, A. pancicii was subjected to phytochemical analysis of EO and Es and biological investigation of Es and isolated compounds for the first time. The results of chemical profiling of EO from aerial parts showed that sesquiterpenoides were the most abundant, although bornyl acetate was the main constituent. MEs revealed 52 compounds in aerial parts and 53 in the roots, mostly coumarins. The study was completed by isolation and identification of a new chromone 5’–acetylcnidimol A and six known furanocoumarins from the roots. The EE of aerial parts showed the highest phenolic content and the best antioxidant results. EE and ME of roots were proven to be good in inhibition of bacterial growth. A. pancicii strongly reduced biofilm synthesis and flagella motility of P. aeruginosa PAO1.

ASSOCIATED CONTENT

*S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

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Funding
The authors are grateful to the Ministry of Education, Science and Technological Development
of the Republic of Serbia for financial support (Grants No. 173029 and 172053).

Notes
The authors declare no competing financial interest.

Abbreviations Used
ABTS, 2,2’–azino–bis(3–ethylbenzothiazoline–6–sulphonic acid); ACN, acetonitrile; AE, aqueous extract; BCB, β–carotene bleaching; BHA, 3–tert–butyl–4–hydroxyanisole; CFU, colony forming units; DCM, dichloromethane; DMSO, dimethylsulfoxide; DPPH, 2,2–
dyphenyl–1–picrylhydrazyl; EE, ethanol extract; EO, essential oil; Es, extrats; ESI, electrospray ionization interface; GC–FID, gas chromatography–flame ionization detector; GC–MS, gas chromatography–mass spectrometry; HPLC–DAD/ESI–ToF–MS, high pressure liquid chromatography–diode array detector/time–of–flight mass spectroscopy; HS–GC–MS, headspace gas chromatography–mass spectrometry; INT, p–iodonitrotetrazolium violet color; LB, Luria–Bertani; MA, Malt agar; MBC, minimum bactericidal concentration; ME, methanol.
extract; MFC, minimum fungicidal concentration; MH, Mueller–Hinton agar; MIC, minimum inhibitory concentrations; Mt., mountain; OD, optical density; PBS, phosphate buffered saline; QS, quorum sensing; SD, standard deviation; TFC, total flavonoid content; TLC, thin layer chromatography; \( t \)-OMe–oxypeucedanin hydrate, tert–O–methyl–oxypeucedanin hydrate; TPC, total phenolic content; TSB, tryptic soy broth.

**Author Contributions**

K. M. and A. Dž. initiated the research concept. K. M. and S. T. wrote the manuscript. P. M. and V. M. organized plant material collection. V. M. and K. M. collected and prepared plant material. M. R., A. Dž. and K. M. performed GC–MS/GC–FID analysis of essential oil. S. T. and Ž. Š. did the chemical analysis of metanolic extracts, isolated and identified compounds. N. T. conducted the NMR analysis. V. T. guided HPLC–DAD/ESI–ToF–MS analysis. K. M. performed the experiments of biological activity determination. K. M. and A. Ć. prepared and analyzed anti–QS activity of the samples and statistically analyzed the data. A. Dž. and P. M. revised the manuscript.

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Table 1. Yields (g) of *A. pancicii* crude Es obtained by ultrasonic extraction

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<tr>
<th></th>
<th>Methanol</th>
<th>Ethanol</th>
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<tr>
<td>Amount 10 g</td>
<td>Aerial parts</td>
<td>Roots</td>
<td>Aerial parts</td>
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<tr>
<td><strong>Yield (g)</strong></td>
<td>1.236</td>
<td>1.905</td>
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Figure 1. The structures of isolated compounds 1–7.

Table 2. Chemical composition of EO of *A. pancicii* aerial parts

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<th>Compounds</th>
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<th>KIL</th>
<th>%</th>
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<td>–</td>
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<td>Compound</td>
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<td>R'</td>
<td>M</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------</td>
<td>------</td>
<td>-----</td>
</tr>
<tr>
<td>3-(p)-Menthene</td>
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<td>(\alpha)-Terpinene</td>
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<td>Isoledene</td>
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**R** refers to the retention time in minutes, **R'** refers to the relative retention time, and **M** refers to the mass of the compound.
<table>
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<th>Compound</th>
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Table 4. Preliminary LC–MS analysis of chemical composition of *A. pancicii* roots ME

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<td>C₂₀H₂₀O₉</td>
<td>Nodakenin²⁴</td>
</tr>
<tr>
<td>8.251</td>
<td>515.1203 [M – H]⁻</td>
<td>C₂₅H₂₃O₁₂</td>
<td>Isochlorogenic acid A²⁴³</td>
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<tr>
<td>8.749</td>
<td>365.0440 [M – H]⁻</td>
<td>C₂₃H₁₉O₅</td>
<td>Xanthoangelol C²²</td>
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<tr>
<td>10.609</td>
<td>305.1020 [M + H]⁺</td>
<td>C₁₅H₁₀O₅</td>
<td>Oxypeucedanin hydrate²³, Heraclenol²⁴</td>
</tr>
<tr>
<td>10.613</td>
<td>349.0953 [M + HCO₂]⁻</td>
<td>C₁₆H₁₀O₆</td>
<td>Oxypeucedanin hydrate²³, Heraclenol²⁴</td>
</tr>
<tr>
<td></td>
<td>339.0657 [M + Cl]⁻</td>
<td>C₁₆H₁₀O₆</td>
<td>Oxypeucedanin hydrate²³, Heraclenol²⁴</td>
</tr>
<tr>
<td>11.678</td>
<td>275.0936 [M – H]⁻</td>
<td>C₁₅H₁₀O₅</td>
<td>Hamaudol²⁵</td>
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*Mass accuracy within 5 ppm
<table>
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<tr>
<th>m/z</th>
<th>Retention Time</th>
<th>Mass (Da)</th>
<th>Formula</th>
<th>Name</th>
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<tr>
<td>12.573</td>
<td>277.1078 [M + H]^+</td>
<td>C_{18}H_{34}O_{5}</td>
<td>Pinellie acid$^56$</td>
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<tr>
<td>12.630</td>
<td>329.2356 [M – H]^-</td>
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<tr>
<td>13.026</td>
<td>379.1761 [M + H]^+</td>
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<tr>
<td>13.022</td>
<td>423.1669 [M + HCO_2]^+</td>
<td>C_{20}H_{36}O_{7}</td>
<td>Angelol C, E, F$^{59}$, L$^{51}$, I$^{60}$</td>
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<tr>
<td></td>
<td>413.1389 [M + Cl]^+</td>
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<td>\textbf{---}</td>
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<tr>
<td>13.026</td>
<td>361.1645 [M + H]^+</td>
<td>C_{19}H_{36}O_{7}</td>
<td>Edulisin IV$^{61}$</td>
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<tr>
<td>13.495</td>
<td>379.1750 [M + H]^+</td>
<td>C_{20}H_{36}O_{7}</td>
<td>Angelol C, E, F$^{59}$, L$^{51}$, I$^{60}$</td>
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<td>13.752</td>
<td>319.1297 [M + H]^+</td>
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<td>13.719</td>
<td>363.1097 [M + HCO_2]^+</td>
<td>C_{17}H_{18}O_{5}</td>
<td>t-OMe–oxyypeucedanin hydrate$^{53}$, 3’–O–Acetylhamaudol$^{65}$</td>
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<td>353.0811 [M + Cl]^+</td>
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<td>13.752</td>
<td>287.0878 [M + H]^+</td>
<td>C_{16}H_{14}O_{5}</td>
<td>Heraclenin$^{55}$, Isooxyypeucedanin$^{56}$, Oxyypeucedanin$^{53}$</td>
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<tr>
<td>14.699</td>
<td>249.1484 [M + H]^+</td>
<td>C_{15}H_{20}O_{3}</td>
<td>Bisabolangelone$^{63}$</td>
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<td>15.046</td>
<td>287.0911 [M + H]^+</td>
<td>C_{16}H_{14}O_{5}</td>
<td>Pabulenol$^{66}$</td>
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<td>15.087</td>
<td>319.1176 [M + H]^+</td>
<td>C_{17}H_{16}O_{6}</td>
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<td>15.086</td>
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<td>15.576</td>
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<td>Osthenol$^{53}$</td>
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<td>15.718</td>
<td>323.0686 [M+H+2]^+</td>
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<td>16.561</td>
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<td>7–Methoxy–5–prenyloxy–coumarin$^{44}$</td>
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<td>16.826</td>
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<td>C_{21}H_{25}O_{7}</td>
<td>Ostruthol$^{66}$, Tomazin$^{54}$</td>
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<tr>
<td>17.300</td>
<td>389.1594 [M + H]^+</td>
<td>C_{21}H_{26}O_{7}</td>
<td>Suksdorfin$^{78}$</td>
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<td>17.340</td>
<td>433.1513 [M + HCO_2]^+</td>
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<tr>
<td>Position</td>
<td>δC, multiplicity</td>
<td>δH multiplicity</td>
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<td>NOESY (H→H)</td>
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<td>(J in Hz)</td>
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<td>166.6, qC</td>
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<td>3</td>
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<tr>
<td>4</td>
<td>182.3, qC</td>
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*Mass accuracy within 5 ppm

**Table 5. NMR data of compound 1**
<table>
<thead>
<tr>
<th>Assay/ A. <em>pandurata</em> Es/Standards</th>
<th>Total phenolic contents</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPC 1 mg/mL (mg GA/g of DE)</td>
<td>TFC 1 mg/mL (mg QE/g of DE)</td>
</tr>
<tr>
<td>ME Aerial parts</td>
<td>72.77 ± 0.00$^c$</td>
<td>35.15 ± 0.00$^a$</td>
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<tr>
<td>Roots</td>
<td>66.68 ± 0.00$^d$</td>
<td>4.46 ± 0.00$^e$</td>
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<tr>
<td>EE Aerial parts</td>
<td>143.99 ± 0.01$^a$</td>
<td>31.39 ± 0.01$^a$</td>
</tr>
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</table>

Table 6. Results of TPCs, TFCs and antioxidant activity of *A. pandurata* Es and standards (means ± SD)
<table>
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<tr>
<th></th>
<th>Roots</th>
<th>4.00 ± 0.00(^c)</th>
<th>0.47 ± 0.00(^c)</th>
<th>1.06 ± 0.01(^b)</th>
<th>5.54 ± 0.00(^c)</th>
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<tbody>
<tr>
<td></td>
<td>Aerial AE parts</td>
<td>84.81 ± 0.00(^c)</td>
<td>9.54 ± 0.00(^b)</td>
<td>0.28 ± 0.01(^b)</td>
<td>0.98 ± 0.01(^b)</td>
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<tr>
<td></td>
<td>Roots</td>
<td>54.37 ± 0.00(^d)</td>
<td>6.38 ± 0.01(^c)</td>
<td>0.41 ± 0.01(^c)</td>
<td>0.64 ± 0.00(^d)</td>
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<tr>
<td>Standards</td>
<td>n.d.</td>
<td>n.d.</td>
<td>BHA 0.13 ± 0.01(^a)</td>
<td>QE 2.75 ± 0.00(^a)</td>
<td>BHA 1.22 ± 0.02(^a)</td>
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Values with different indicated letters in the same column mean significant difference (p < 0.05).

n.d. – not determined
Table 7. Results of antibacterial activity of A. pancicii Es, isolated compounds and streptomycin in mg/mL (means ± SD)

<table>
<thead>
<tr>
<th>A. pancicii Es/Standard</th>
<th>B. cereus</th>
<th>M. flavus</th>
<th>L. monocytogenes</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
<th>E. coli</th>
<th>E. cloacae</th>
<th>S. tiphymurium</th>
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</thead>
<tbody>
<tr>
<td><strong>Aerial</strong></td>
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<td></td>
</tr>
<tr>
<td>MIC</td>
<td>0.50 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.00 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.00 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MBC</td>
<td>1.00 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.00 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>MIC</td>
<td>0.20 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.50 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
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<td>0.50 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.00 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>7.00 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.00 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>3.00 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>8.00 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.00 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>n.i.</td>
<td>2.00 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>n.i.</td>
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<td>16.00 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.00 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.i.</td>
<td>n.i.</td>
<td>16.00 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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<td>16.00 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.00 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.00 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MBC</td>
<td>16.00 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.i.</td>
<td>16.00 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.00 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.00 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>n.i.</td>
<td>2.00 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.00 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ostruthol</td>
<td>16.00 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.00 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.00 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.00 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.00 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.00 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MBC</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Oxypeucedanin hydrate</td>
<td>n.i.</td>
<td>2.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MBC</td>
<td>0.50 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.00 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoimperatorin</td>
<td>4.00 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.00 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.00 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.00 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.00 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.00 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MBC</td>
<td>8.00 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.i.</td>
<td>4.00 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.00 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.00 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.i.</td>
<td>n.i.</td>
<td>8.00 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetyl cnidimol A</td>
<td>4.00 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.00 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.00 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.00 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.00 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.00 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MBC</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.09 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.17 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MBC</td>
<td>0.37 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.74 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.49 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with different indicated letters in the same line mean significant difference (p < 0.05).

n.i. – not identified
Table 8. Results of antifungal activity of *A. pancicii* Es and fluconazole in mg/mL (means ± SD)

<table>
<thead>
<tr>
<th>Fungi/ A. pancicii Es/Standard</th>
<th>C. albicans</th>
<th>T. viride</th>
<th>P. ochrochloron</th>
<th>P. funiculosum</th>
<th>A. fumigatus</th>
<th>A. versicolor</th>
<th>A. ochraceus</th>
<th>A. niger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial</td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
<td>MFC</td>
<td>MFC</td>
</tr>
<tr>
<td>ME</td>
<td>3.00 ± 0.03a</td>
<td>6.00 ± 0.04b</td>
<td>6.00 ± 0.02b</td>
<td>6.00 ± 0.05b</td>
<td>6.00 ± 0.08b</td>
<td>6.00 ± 0.05b</td>
<td>8.00 ± 0.07c</td>
<td>8.00 ± 0.06c</td>
</tr>
<tr>
<td>parts</td>
<td>8.00 ± 0.02a</td>
<td>10.00 ± 0.02a</td>
<td>10.00 ± 0.10a</td>
<td>8.00 ± 0.08a</td>
<td>12.00 ± 0.07a</td>
<td>8.00 ± 0.08a</td>
<td>16.00 ± 0.04b</td>
<td>16.00 ± 0.02b</td>
</tr>
<tr>
<td>MIC</td>
<td>4.00 ± 0.07a</td>
<td>6.00 ± 0.03b</td>
<td>6.00 ± 0.02b</td>
<td>3.00 ± 0.05b</td>
<td>6.00 ± 0.04b</td>
<td>6.00 ± 0.05b</td>
<td>6.00 ± 0.07b</td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>14.00 ± 0.04b</td>
<td>10.00 ± 0.08b</td>
<td>10.00 ± 0.05b</td>
<td>4.00 ± 0.02a</td>
<td>10.00 ± 0.08b</td>
<td>12.00 ± 0.05b</td>
<td>12.00 ± 0.01b</td>
<td></td>
</tr>
<tr>
<td>MFC</td>
<td>16.00 ± 0.04b</td>
<td>12.00 ± 0.02a</td>
<td>12.00 ± 0.11a</td>
<td>8.00 ± 0.07a</td>
<td>12.00 ± 0.02a</td>
<td>12.00 ± 0.05a</td>
<td>16.00 ± 0.07b</td>
<td>16.00 ± 0.05b</td>
</tr>
<tr>
<td>Aerial</td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
<td>MFC</td>
<td>MFC</td>
</tr>
<tr>
<td>EE</td>
<td>6.00 ± 0.02a</td>
<td>6.00 ± 0.11a</td>
<td>8.00 ± 0.08a</td>
<td>6.00 ± 0.04a</td>
<td>8.00 ± 0.00a</td>
<td>6.00 ± 0.03a</td>
<td>6.00 ± 0.03a</td>
<td>6.00 ± 0.01a</td>
</tr>
<tr>
<td>parts</td>
<td>8.00 ± 0.08a</td>
<td>10.00 ± 0.07b</td>
<td>10.00 ± 0.03b</td>
<td>8.00 ± 0.02a</td>
<td>10.00 ± 0.11b</td>
<td>8.00 ± 0.02a</td>
<td>12.00 ± 0.07b</td>
<td>12.00 ± 0.03b</td>
</tr>
<tr>
<td>MIC</td>
<td>14.00 ± 0.05a</td>
<td>12.00 ± 0.12b</td>
<td>14.00 ± 0.05b</td>
<td>10.00 ± 0.07a</td>
<td>10.00 ± 0.13a</td>
<td>10.00 ± 0.08a</td>
<td>14.00 ± 0.03b</td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>18.00 ± 0.08b</td>
<td>14.00 ± 0.05a</td>
<td>&gt;18.00 ± 0.08b</td>
<td>&gt;18.00 ± 0.05b</td>
<td>&gt;18.00 ± 0.10b</td>
<td>&gt;18.00 ± 0.09b</td>
<td>&gt;18.00 ± 0.07b</td>
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</tr>
<tr>
<td>MFC</td>
<td>14.00 ± 0.01a</td>
<td>12.00 ± 0.07b</td>
<td>10.00 ± 0.05a</td>
<td>10.00 ± 0.03a</td>
<td>10.00 ± 0.02b</td>
<td>10.00 ± 0.05a</td>
<td>14.00 ± 0.02a</td>
<td>10.00 ± 0.02a</td>
</tr>
<tr>
<td>EE</td>
<td>16.00 ± 0.03a</td>
<td>16.00 ± 0.08b</td>
<td>18.00 ± 0.09b</td>
<td>16.00 ± 0.10a</td>
<td>18.00 ± 0.08b</td>
<td>18.00 ± 0.03b</td>
<td>&gt;18.00 ± 0.06b</td>
<td>&gt;18.00 ± 0.11b</td>
</tr>
<tr>
<td>parts</td>
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<td>1.00 ± 0.01c</td>
<td>1.00 ± 0.07c</td>
<td>0.25 ± 0.00b</td>
<td>0.50 ± 0.02a</td>
<td>0.13 ± 0.02a</td>
<td>0.50 ± 0.00b</td>
<td>0.25 ± 0.03b</td>
</tr>
<tr>
<td>MIC</td>
<td>0.03 ± 0.00a</td>
<td>1.50 ± 0.03d</td>
<td>1.50 ± 0.03d</td>
<td>0.50 ± 0.05b</td>
<td>1.00 ± 0.02a</td>
<td>0.50 ± 0.03b</td>
<td>1.00 ± 0.05c</td>
<td>1.00 ± 0.01c</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>MFC</td>
<td>MFC</td>
<td>MFC</td>
<td>MFC</td>
<td>MFC</td>
<td>MFC</td>
<td>MFC</td>
<td>MFC</td>
</tr>
</tbody>
</table>

Values with different indicated letters in the same line mean significant difference (p < 0.05).
Table 9. Results of effects of *A. pancicii* Es, isolated compounds and standards (0.5MICs) on *P. aeruginosa* PAO1 twitching and flagella motility and biofilm formation (%)

<table>
<thead>
<tr>
<th>Parameters/ A. pancicii Es/Standards</th>
<th>Colony diameter (mm ± SE)</th>
<th>Flagella diameter (µm)</th>
<th>Colony color</th>
<th>Colony edge</th>
<th>Biofilm formation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5 MIC (% ± SE)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>0.25 MIC (% ± SE)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.125 MIC (% ± SE)</td>
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<tr>
<td><strong>ME</strong></td>
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<td></td>
</tr>
<tr>
<td>Aerial parts</td>
<td>22.67 ± 2.52^a</td>
<td>16 – 56</td>
<td>Light green</td>
<td>Tiny flagella</td>
<td>65.22 ± 1.87^a</td>
</tr>
<tr>
<td>Roots</td>
<td>20.67 ± 8.02^ab</td>
<td>40 – 96</td>
<td>Green</td>
<td>Tiny flagella</td>
<td>52.43 ± 0.50^c</td>
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<tr>
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<td></td>
<td></td>
<td>40.38 ± 0.86^b</td>
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<tr>
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<td></td>
<td></td>
<td>34.54 ± 0.50^b</td>
</tr>
<tr>
<td><strong>EE</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Aerial parts</td>
<td>23.67 ± 6.51^b</td>
<td>56 – 128</td>
<td>Green</td>
<td>Regular flagella</td>
<td>53.58 ± 2.34^c</td>
</tr>
<tr>
<td>Roots</td>
<td>9.33 ± 1.53^a</td>
<td>/</td>
<td>Light green</td>
<td>/</td>
<td>46.98 ± 2.37^b</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>25.66 ± 0.58^ab</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>17.36 ± 1.58^a</td>
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<td></td>
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<tr>
<td>Aerial parts</td>
<td>23.00 ± 12.12^b</td>
<td>40 – 160</td>
<td>Green</td>
<td>Regular flagella</td>
<td>62.26 ± 2.47^c</td>
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<tr>
<td>Roots</td>
<td>11.00 ± 1.00^a</td>
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<td>Light green</td>
<td>/</td>
<td>72.83 ± 0.85^c</td>
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<td>74.53 ± 2.21^c</td>
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<td></td>
<td>69.62 ± 1.06^cd</td>
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<tr>
<td><em>tert</em>-O–methyl oxypeucedanin hydrate</td>
<td>16.66 ± 7.64^a</td>
<td>32</td>
<td>White</td>
<td>Tiny and reduced flagella</td>
<td>32.47 ± 1.07^b</td>
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<td></td>
<td>21.80 ± 0.44^a</td>
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<tr>
<td>Oxypeucedanin</td>
<td>8.66 ± 4.04^a</td>
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<td>White</td>
<td>/</td>
<td>10.60 ± 0.53^a</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>49.46 ± 0.93^bc</td>
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<td>/</td>
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<tr>
<td>Saxalin</td>
<td>23.33 ± 6.51^b</td>
<td>112</td>
<td>White</td>
<td>Reduced flagella</td>
<td>52.17 ± 0.91^c</td>
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<td></td>
<td></td>
<td></td>
<td>32.40 ± 1.87^b</td>
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<td></td>
<td>57.88 ± 3.53^c</td>
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<tr>
<td>Ostruthol</td>
<td>23.00 ± 5.57^b</td>
<td>160</td>
<td>White</td>
<td>Regular flagella</td>
<td>30.64 ± 1.08^b</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.06 ± 1.03^a</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>40.35 ± 0.81^b</td>
</tr>
<tr>
<td>Oxypeucedanin</td>
<td>15.33 ± 1.53^a</td>
<td>80</td>
<td>White</td>
<td>Tiny flagella</td>
<td>41.44 ± 2.50^b</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>37.30 ± 0.68^b</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>2.72 ± 0.23^a</td>
</tr>
<tr>
<td>Chemical</td>
<td>Value 1 ± SD</td>
<td>Value 2</td>
<td>Value 3 ± SD</td>
<td>Value 4 ± SD</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------</td>
<td>----------</td>
<td>--------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>Isoimperatorin</td>
<td>46.66 ± 5.77c</td>
<td>48</td>
<td>White Tiny</td>
<td>71.60 ± 1.93c</td>
<td>57.68 ± 2.01c</td>
</tr>
<tr>
<td>Acetyl cnidimol A</td>
<td>28.00 ± 7.55b</td>
<td>280</td>
<td>White Regular</td>
<td>35.05 ± 1.02b</td>
<td>–</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>11.00 ± 1.00a</td>
<td>24 – 56</td>
<td>Green Tiny</td>
<td>69.16 ± 0.65c</td>
<td>56.46 ± 0.46c</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>13.33 ± 5.03a</td>
<td>16 – 56</td>
<td>Green Regular</td>
<td>49.40 ± 0.46bc</td>
<td>70.97 ± 0.36c</td>
</tr>
<tr>
<td>Control (10⁹ CFU/mL)</td>
<td>12.00 ± 1.00a</td>
<td>56 – 80</td>
<td>Light green</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

Values with different indicated letters in the same column mean significant difference (p < 0.05).

Biofilm formation values were calculated as: ((mean A₆₂₀ control well)/(mean A₆₂₀ treated well)/mean A₆₂₀ control well) x 100.

Values are expressed as means ± SD.
Figure 2. Light microscopy of colony edges of *P. aeruginosa* in twitching motility, grown in the presence of 0.5 MICs of *A. pancicii* Es, isolated compounds and antibiotics. The bacterial colonies grown with the presence of Es (A–F); The bacterial colonies grown with the presence of *A. pancicii* compounds (G–M); *P. aeruginosa* colony in the presence of streptomycin had reduced protrusion (N); *P.
*P. aeruginosa* colony in presence of ampicillin with regularly formed protrusions (O); *P. aeruginosa* produced a flat, widely spread, irregularly shaped colony in the absence of tested samples (P); Magnification: (A–D)×100.

**Figure 3.** Reduction of pyocyanin production of *P. aeruginosa* PAO1 by *A. pancicii* Es, isolated compounds and antibiotics tested at 0.5MICs (mg/mL).