JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

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

Downloaded from http://pubs.acs.org on November 13, 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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24 **ABSTRACT**: The essential oil, different extracts and isolated compounds of *Angelica pancicii*
25 Vandas (Apiaceae) were investigated for the first time. The GC–FID and GC–MS analyses
26 revealed sesquiterpenoids as the main constituents of *A. pancicii* essential oil of aerial parts with
27 bornyl acetate (8.08%), *n*–octanol (5.82%), kessane (4.26%) and *β*–selinene (4.26%) as the main
28 constituents. Analysis of methanol extracts, using HPLC–DAD/ESI–ToF–MS system, showed a
29 total of 52 compounds in the aerial parts and 53 in the roots, indicated coumarins as the main
30 constituents. In addition, new chromone (**1**) and six known furanocoumarins (**2**–**7**) were isolated
31 from the roots and structurally elucidated by combined spectroscopic methods. The aerial parts
32 extracts exhibited higher polyphenolic contents and antioxidant activity evaluated by three
33 radical scavenging assays. Using micro–well dilution method, the strongest antibacterial activity
34 profiles were determined for ethanol and methanol root extracts (minimum bactericidal
35 concentrations (MBCs) = 0.25–3.00 mg/mL), which were comparable to the activity of
36 streptomycin (MBCs = 0.34–1.24 mg/mL), while the strongest antibacterial compound of *A.*
37 *pancicii* was oxypeucedanin hydrate (MBCs = 0.50–8.00 mg/mL). Antifungal potential was in
38 moderate extent and the highest activity was obtained for roots methanol extract (minimum
39 fungicidal concentrations (MFCs) = 4.00–14.00 mg/mL). Tested sub–minimum inhibitory
40 concentrations (subMICs) of the extracts and isolated compounds inhibited selected
41 *Pseudomonas aeruginosa* PAO1 virulence determinants. The most reduced growth of *P.*
42 *aeruginosa* colony was in the presence of isolated oxypeucedanin. Ethanol (17.36–46.98%) and
43 methanol (34.54–52.43%) roots extracts showed higher anti–biofilm activity compared to
44 streptomycin (49.40–88.36%) and ampicillin (56.46–92.16%).

45

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

48

49 **INTRODUCTION**

50

51 The genus *Angelica* L. (Apiaceae) consists of about 50 species of tall, biennial and
52 perennial herbs, native to temperate and subarctic regions of the Northern Hemisphere.[1] *Angelica*
53 *pancicii* Vandas is a perennial, endemic species of Balkan peninsula.[2] The genus *Angelica* is well
54 known for many nutritional and medicinal properties. More than half of *Angelica* species have
55 pharmaceutical and ethno–medicinal utility and some of them are included in several national
56 and European pharmacopoeias.[3] Many angelicas are edible and a few are cultivated for food and
57 alcohol industry usage. *Angelica archangelica* is traditionally used in the Far East and many
58 parts of Western world. It was used by the native Sami population of northern Scandinavia for
59 preservation of reindeer milk, preparation of cheese, as a natural sweetener and substitute for
60 tobacco. Today, as World Economic Plant, it is grown commercially in many European countries
61 for application of its aromatic root. The essential oil of the roots is well known as vanilla–like
62 flavoring in commercial liqueurs, sweets and honey, while leaves are added to cooked fruit
63 dishes, soups, fish or poultry.[4] *A. sinensis* is also in culinary usage in China.[5] In the US, this plant
64 is marketed as a dietary supplement.[6] Fresh leaves of herb *A. keiskei* are widely used for
65 preparation of green juice and health–promoting food in Japan,[7] while it is widely planted in
66 Taiwan and consumed as a vegetable in many restaurants.[8] *A. dahurica* is also frequently used as
67 herbal ingredient in functional food and folk medicine,[9] while highly valued aromatic plant of
68 the Himalaya *A. glauca* is considered as useful cure for treatment of the gastrointestinal
69 disorders.[10] Many studies have confirmed various pharmaceutical properties of *Angelica* species,
70 which are rich source of metabolites (essential oils, coumarins, acetylenic compounds,

71 chalcones, sesquiterpenes, polysaccharides, etc.) with broad spectrum of biological activities.
72 Coumarins, the most characteristic chemical markers of the genus, could be considered as
73 leading active principles of angelicas.[11,12] As regards *A. pancicii*, only essential oil of the plant
74 was characterized so far, show in high content of monoterpenoids.[13] From the genus *Angelica,*
75 only *A. dahurica* (essential oil),[14,15] and *A. sinensis* (roots extracts)[16] were tested for anti–quorum
76 sensing (QS) activity on *Pseudomonas aeruginosa* so far. A promising approach to combat this
77 bacterium is to target its communication system by anti–QS agents through disruption of biofilm
78 formation, flagella system, production of toxins, virulence enzymes *etc*. The final result is
79 pathogen more susceptible to the antibiotics. Some popular, edible species, e.g. culinary spice
80 *Curcuma longa*, were showed to attenuate the virulence of this bacillus on this way.[17]

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

90

91 **MATERIALS AND METHODS**

92

93 **Chemicals and Apparatus**. Analytical grade solvents were provided from Zorka pharma
94 (Šabac, Serbia) and before using for extraction and chromatographic separation were freshly
95 distilled. For LC–MS and semi–preparative HPLC analyses, acetonitrile purchased from Merck
96 KG (Darmstadt, Germany), formic acid (85% purity) from Lach–Ner, s.r.o. (Neratovice, Czech
97 Republic) and Milli Q water 18.2 MΩ–cm, obtained from a Millipore Simplicity 185 purification
98 system were used. For dry–column flash chromatography, silica gel 0.08 mm (Merck) was used.
99 Analytical TLC was carried out on silica gel 60 GF254 20 × 20 cm plates, layer thickness 0.25
100 mm (Merck). Preparative HPLC was performed on an Agilent 1100 series instrument equipped
101 with a DAD, using a Zorbax Eclipse XDBC–18 column (250 mm × 9.4 mm, 5 μm). Mass
102 spectral (HR–ESI–MS) data were obtained from an Agilent Technologies 6210 time–of–flight
103 LC/MS system. 1D and 2D NMR spectra were recorded on a Bruker Avance III 500
104 spectrometer (500.26 MHz for ^{1}H and 125.80 MHz for ^{13}C nuclei) equipped with 5mm broad-
105 band probehead (BBO). The spectra were measured at room temperature (298K) in $CDCl_3$ for all
106 compounds, with addition of 4 drops of MeOD only for compound **1** in order to increase its
107 solubility. All spectra were referenced to the residual solvent's signal (7.26 ppm and 77 ppm for
108 ^{1}H and ^{13}C respectively). For 2D spectra, H-H COSY, H-H NOESY, H-C HSQC and H-C
109 HMBC, the standard Bruker pulse sequences were applied (cosygpmfqf, noesygpph,
110 hsqcetgpsi2, hmbcgplpndqf). IR spectra were recorded on a ThermoScientific Nicolet 6700 FT–
111 IR spectrometer using a capillary film technique. Optical rotations were measured on a Rudolph
112 Research Analytical AUTOPOL IV automatic polarimeter. Elemental analysis was performed on
113 a Vario EL III C,H,N,S/O elemental analyzer (Elementar). The spectrophotometric
114 measurements were performed using JENWAY 6306 UV/Vis spectrophotometer.

115 Gallic acid (GA) (98%), 3–tert–butyl–4–hydroxyanisole (BHA), 2,2–dyphenyl–1–
116 picrylhydrazyl (DPPH), Folin–Ciocalteu phenol reagent, potassium acetate (≥99%), aluminum
117 trinitrate nonahydrate (≥98%), dimethyl sulfoxide dried and *ß*–carotene (analytical grade) and *p*–
118 iodonitrotetrazolium violet color (INT) were obtained from Sigma–Aldrich Co., St Louis, MQ,
119 USA. Sodium carbonate anhydrous (analytical grade) was obtained from Centrohem doo (Stara
120 Pazova, Serbia). Potassium peroxidisulphate (≥99%), L(+)–ascorbic acid, Tween 80 and linoleic
121 acid (analytical grade) were obtained from Acros organics, Fisher Scientific UK Ltd.,
122 Loughborough, Leicestershire, UK. 2,2'–Azino–bis(3–ethylbenzothiazoline–6–sulphonic
123 acid(ABTS) and quercetin hydrate (QE) (≥98%) were purchased from TCI Europe NV,
124 Boerenveldsweg, Belgium. Mueller–Hinton Agar (MH), Malt Agar (MA) and Tryptic Soy Broth
125 (TSB) were obtained from the Institute of Immunology and Virology, Torlak (Belgrade, Serbia),
126 while streptomycin and ampicillin solutions (100 μg/mL) from Hyclone (Logan, Utah, USA).
127 Dimethylsulfoxide (DMSO, ≥99.9%) was purchased from Merck KGaA, Germany. Antimicotic
128 Diflucan (containing 50 mg fluconazole) was obtained from Pfizer PGM, Pocesur–Cisse, France.
129 **Plant Material**. Plant material in pre–flowering stage was collected at Mt. Pelister, a
130 national park which is in the surrounding of Bitola city in Republic of Macedonia (GPS: N
131 41°01’58”; E 21°11’59”) in July, 2013 and 2014. It was determined as *Angelica pancicii* Vandas
132 by Prof. V. S. Matevski. Voucher specimens of *A. pancicii* (BU16776 (2013) and BU16672
133 (2014)) are deposited at the Herbarium of the Institute of Botany and Botanical Garden
134 “Jevremovac”, (BEOU), Faculty of Biology, University of Belgrade, Serbia.
135 **EO Isolation**. Greenish EO of *A. pancicii* was isolated from dry plant material (200 g) by
136 hydrodistilation (3h) using Clevenger type apparatus.[18] Obtained oil (nonquantifiable yield) was
137 refrigerated in a sealed vial prior to the chemical analysis.

138 **Extraction Procedure 1**. Methanol (ME), ethanol (EE) and aqueous extracts (AE) of *A.*
139 *pancicii* aerial parts and roots collected in 2013 were obtained by the ultrasonic (US) extraction
140 conducted in dark conditions. The procedure for US extraction was followed according to
141 modified method described by Džamić et al.[19] 10g of each grounded sample were extracted for
142 24 h with 200 mL of listed solvents using an ultrasonic apparatus for the first and the last hour of
143 extraction. Afterwards, the filtration was carried out using Whatman filter paper No1. MEs and
144 EEs were evaporated under reduced pressure at maximum temperature of 40 ℃, while frozen
145 AEs were lyophilized. Upon measurements (Table 1), obtained Es were packed in glass bottles
146 and kept under refrigeration until further utilization.

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

150 **GC Analysis with FID and MS Detection.** The analysis of the oil was carried out on a
151 GC HP–5890 II apparatus, equipped with split–splitless injector, attached to HP–5 column (25 m
152 × 0.32 mm, 0.52 μm film thickness, Agilent Technologies, Waldbronn, Germany) and fitted to
153 FID. Carrier gas flow rate (H_2) was 1 mL/min, split ratio 1:30, injector temperature was 250 °C,
154 detector temperature 300 °C; column temperature was linearly programmed from 40–240 °C (at
155 rate of 4 °/min). The same conditions were employed for Gas Chromatography–Mass
156 Spectrometry (GC–MS) analysis; HP G 1800C Series II GCD system equipped with HP–5MS
157 column (30 m × 0.25 mm, 0.25 μm film thickness, Agilent Technologies, Waldbronn, Germany)
158 was used. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV) in
159 *m/z* range 40–400. Identification of EO components was accomplished by matching mass
160 spectral data with those held in Wiley 275 mass spectral library and comparison of obtained

161 retention indexes with Adams 0.4. RI library data[22] using AMDIS software. For the purpose of
162 relative abundance determination, percentages of peak area of compounds relative to the total
163 area obtained by Flame Ionization Detector (FID) were used.

164 **LC Analysis with DAD and MS Detection**. The analysis of the MEs of the roots and the
165 aerial parts obtained by *Extraction procedure 1* were performed using
166 HPLC–DAD/ESI–ToF–MS system consisting of an HPLC instrument Agilent 1200 Series
167 (Agilent Technologies, Waldbronn, Germany) equipped with a degasser, a binary pump, an
168 auto–sampler, a termostated column compartment and a diode array detector (DAD) and coupled
169 with a 6210 Time–of–Flight LC/MS system (Agilent Technologies, Santa Clara, California,
170 USA) via an electro spray ionization (ESI) interface. Immediately before analysis, the dry
171 residues of MEs were re–dissolved in 1 mL acetonitrile–methanol (95:5) and samples (c=10.0
172 mg/mL) were filtered through Captiva Premium Syringe Filter Agilent Technologies (0.45 μm ×
173 25 mm) and, in a volume of 5 μL, injected into a Zorbax Eclipse Plus C18 (150 mm × 4.6 mm i.
174 d.; 1.8 μm) column, maintained at 40 °C. The mobile phase was a mixture of solvent A (0.20%
175 formic acid in water) and solvent B (acetonitrile) according to a combination of isocratic and
176 gradient modes of elution: 0–1.5 min, 95% A, 1.5–26 min, 95–5% A, 26–35 min, 5% A, 36–41
177 min, 95% A, at a flow rate of 1.40 mL/min. Detection was accomplished using DA detector and
178 storing the signals in the wavelength range from 190–650 nm. The HPLC effluent was directed
179 into the atmospheric pressure ESI ion source of the mass spectrometer. The eluted compounds
180 were mixed with nitrogen in the heated nebulizer interface and the polarity was tuned to
181 positive/negative. An adequate calibration of the ESI parameters (capillary voltage, gas
182 temperature, nebuliser pressure, and fragmentor voltage) was required to optimise the response
183 and to obtain a high sensitivity of the molecular ion. The MS conditions were as follows:

184 capillary voltage, 4000 V; gas temperature, 350 °C; drying gas, 12 mL/min; nebuliser pressure,
185 45 psig; fragmentor voltage, 140 V; mass range, 100–2000 *m/z*.[23] A personal computer system
186 running Mass Hunter Workstation software was used for data acquisition and processing. The
187 Molecular Feature Extractor of Mass Hunter Workstation was used to predict chemical formulas.

188 **Isolation Procedure 1**. A part of roots ME (1.5g) obtained by *Extraction procedure 1*
189 was dissolved in destilated H_2O and re–extracted with DCM (3 × 100 mL), ethyl–acetate (3 ×
190 100 mL) and butanol, consecutively. Organic phases were drying with anhydrous Na_2SO_4,
191 filtrated, evaporated and analysed by TLC, LC/MS and NMR. DCM fraction (649.2 mg) was
192 chosen for further separation by silica gel dry–flash column chromatography (80g, 18 × 2.2 cm^2).
193 The elution started with petrol (4.9 mg) and continued as follows: Et_2O (fraction **F2**, 260.4 mg),
194 Et_2O–DCM 1:1 (**F3**, 127.9 mg), DCM (10.7 mg), DCM–MeOH 99:1 (1.5 mg), 95:5 (1.1 mg),
195 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),
196 methanol (5.8 mg). Each fraction was eluted with 50 mL of the solvent system. Fractions **F2**, **F3**
197 and **F7** were further fractionated by semi–preparative HPLC–DAD to isolate pure compounds
198 using a 0.02% HCOOH/ACN (acetonitrile) elution system with a flow rate of 4 mL/min and the
199 following gradient program: 0–2 min, 50% ACN; 2–17 min, 50–65% ACN; 17–20 min, 65%
200 ACN. The detection wavelengths were 220, 260, 280, 320 and 360 nm. A part of fraction **F2**
201 (200 mg) was divided into two fractions, yielding coumarin saxalin (**4**, R_t 9.87–10.49 min, 2.583
202 mg) and a mixture of oxypeucedanin and oxypeucedanin hydrate (**3** + **6**, R_t 9.14–9.77 min). Due
203 to the fact that 0.02% HCOOH was used for fractionation, it was assumed that dihydroxide **6** was
204 formed from epoxyde **3** by ring–opening. Thus, a rest of **F2** was purified without HCOOH, with
205 H_2O/ACN elution system and the same gradient program. Oxypeucedanin (**3**, 4.647 mg) was
206 isolated at the same retention time. Applying a semi–preparative HPLC–DAD under the former

207 conditions, coumarins *t*–OMe–oxypeucedanin hydrate (**2**, R_t 7.2–7.7 min, 7.442 mg), saxalin (**4**,
208 R_t 10.1–10.5 min, 3.216 mg), ostruthol (**5**, R_t 11.9–12.4 min, 22.591 mg) and the new chromone
209 5'–acetylcnidimol A (**1**, R_t 8.6–9.2 min, 6.8 mg) were isolated from **F3**, as well as
210 oxypeucedanin hydrate (**6**, 12.959 mg) from **F7**. The structures of isolated compounds are given
211 in Figure 1.

212 **Isolation Procedure 2**. DCM extract obtained by Soxhlet extraction was further
213 separated by semi–preparative HPLC using a H_2O/ACN elution system with a flow rate of 4
214 mL/min and the following gradient program: 0–2 min, 50% ACN; 2–17 min, 50–65% ACN; 17–
215 20 min, 65% ACN. Besides previously isolated compounds, **1** (R_t 8.43–9.09, 11.876 mg), **3** (R_t
216 9.17–9.77, 10.507 mg), **4** (R_t 9.88–10.4 min, 6.219 mg) and **5** (R_t 11.66–12.43 min, 31.076 mg),
217 isoimperatorin was also isolated (**7**, R_t 17.18–17.73 min, 3.180 mg).

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

224 **Antimicrobial Activity**. *Preparation of Stock Solutions of Es and Isolated Compounds.*
225 Crude *A. pancicii* Es obtained by *Extraction Procedure 1* were dissolved in 5% DMSO to obtain
226 stock solutions (20 mg/mL). Purified compounds were dissolved in 5% DMSO in addition of
227 Tween 40 (in concentration of 0.1%) to gain stock solution of 2 mg/mL. Subsequently, different
228 dilutions of these solutions were examined against 8 bacteria, 7 fungi and 1 yeast to determinate
229 their antimicrobial potency.

230 *Microbial Cultures Treated Isolates*. For testing of investigated samples, pure control
231 strains were obtained from mycological laboratory, Department of Plant Physiology, Institute for
232 Biologycal Research "Siniša Stanković", Belgrade, Serbia. The following bacteria were used:
233 *Bacillus cereus* (food isolate), *Micrococcus flavus* (ATCC 10240), *Listeria monocytogenes*
234 (NCTC 7973) and *Staphylococcus aureus* (ATCC 6538) of Gram positive and *Pseudomonas*
235 *aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35210), *Enterobacter cloacae* (human
236 isolate) and *Salmonella typhimurium* (ATCC 13311) of Gram negative bacteria. Listed fungi
237 were used: *Trichoderma viride* (IAM 5061), *Penicillium ochrochloron* (ATCC 9112),
238 *Penicillium funiculosum* (ATCC 10509), *Aspergillus fumigatus* (ATCC 9197), *Aspergillus*
239 *ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger*
240 (ATCC6275) and yeast *Candida albicans* (ATCC 10231). Dilutions of bacterial inocula were
241 cultured on solid MH medium, while fungi were maintained on solid MA medium. The cultures
242 were subcultured once a month and stored at + 4 °C for further usage.[29]

243 *Micro–Well Dilution Assay*. The antimicrobial activity of *A. pancicii* samples was
244 assayed by modified microdilution method described by CLSI[30] and Hanel and Raether.[31] The
245 technique was carried out in sterile 96–well microtiter plates, by adding different 5% DMSO
246 dilutions of Es and isolated compounds into corresponding medium – TSB and MA, for bacteria
247 and fungi, respectively. Prior to experiment, bacterial and fungal inocula were prepared. For
248 bacterial strains, 100 μL of overnight cultures were mixed with 900 μL of medium to obtain the
249 concentration of 1.0×10^8 colony forming units (CFU)/mL. Fungal inocula were prepared by
250 washing spores with sterile 0.85% saline solution (which contains 0.1% Tween 80 (v/v)). The
251 microbial cell suspensions were adjusted with sterile saline to a concentration of approximately
252 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

253 well. Incubation of bacteria at 37 °C lasted for 24 h, and for fungi 72 h at 28 °C. The minimum
254 inhibitory concentrations (MICs) are defined as the lowest concentrations of tested samples,
255 completely inhibiting the growth of used pathogens. The lowest concentrations with no visible
256 growth after serial sub–cultivation, indicating 99.5% killing of the original inoculums, are
257 determined as the minimum bactericidal/fungicidal concentrations (MBCs/MFCs).[31] The growth
258 of tested bacteria was determined by a colorimetric microbial viability assay, based on reduction
259 of a 0.2% INT aqueous solution and compared with positive control for each strain.[30,32] Two
260 repeats were done for each sample. The 5% DMSO streptomycin solution (1 mg/mL) was used
261 as positive control for bacteria. The solution of standard fluconazole (2 mg/mL) was included for
262 fungi. Sterilized distilled water containing 0.02% Tween 80 and 5% DMSO was used as negative
263 control.

264 **Anti–QS Activity**. *Bacterial Strains, Growth Media and Culture Conditions*. For
265 performing the experiments, *Pseudomonas aeruginosa* PAO1 from the collection of the
266 Mycoteca, Institute for Biological Research "Siniša Stanković", Belgrade (Serbia), was used. The
267 strain was routinely grown in Luria–Bertani (LB) medium (1% w/v NaCl, 1% w/v Tryptone,
268 0.5% w/v yeast extract) with shaking (220 rpm) and cultured at 37°C.

269 *Biofilm Formation*. To determinate the effect of *A. pancicii* Es and isolated compounds
270 on *P. aeruginosa* biofilm formation, the method described by Drenkard and Ausubel[33] and
271 Spoering and Lewis[34] with some modifications was followed. The anti–biofilm forming ability
272 of different concentrations (0.125, 0.25 and 0.5 of MICs) of samples was tested, using
273 polystyrene flat–bottomed microtitre 96–well plates. Subsequently after pipetting 100 μL of sub–
274 inhibitory concentrations (subMICs) of tested samples and 100 mL of medium (control), 100 μL
275 of *P. aeruginosa* overnight culture inoculum was added into each well of the plate. The

276 incubation at 37 ºC lasted for 24 h and after that each well was washed twice with sterile
277 phosphate buffered saline (PBS, pH 7.4) and dried. In order to determine the biofilm mass, 0.1%
278 crystal violet was poured and left for 10 min. After drying, 200 μL of 95% ethanol (v/v) was
279 added to solubilise the dye that had stained the biofilm cells. The excess stain was washed off
280 with distilled H_2O. After 10 min, the content of the wells was homogenized and the absorbance
281 at λ = 625 nm was read on a Sunrise™ –Tecan ELISA reader. The experiment was done in
282 triplicate and repeated two times. The values were presented as a mean values ± standard
283 deviation (SD).

284 *Twitching and Flagella Motility*. The cells of *P. aeruginosa*, grown in the presence or the
285 absence of Es and isolated compounds, were washed twice with sterile PBS and re–suspended in
286 PBS at 1×10^8 CFU/mL (optical density (OD) of 0.1 at 660 nm). In brief, the cells were stabbed
287 into a nutrient agar plate with a sterile tooth pick and incubated overnight at 37 °C. Plates were
288 then removed from the incubator and incubated at room temperature for 48 h. Colony edges and
289 the zone of motility were measured with a light microscope.[35,36] 0.5MICs of samples were mixed
290 into 10 mL of molten MH medium and poured immediately over the surface of a solidified LB
291 agar plate as an overlay. The plate was point inoculated with an overnight culture of PAO1 once
292 the overlaid agar had solidified and incubated at 37 °C for 3 days. The extent of swimming was
293 determined by measuring the area of the colony.[37] The experiment was done in triplicate and
294 repeated two times. The colony diameters were measured three times in different direction and
295 values were presented as a mean values ± SD.

296 *Pyocyanin Production*. The quantification of ability of tested Es and isolated compounds
297 to inhibit *P. aeruginosa* pyocyanin production was conducted using the flask assay. *P.*
298 *aeruginosa* overnight culture was diluted to OD_{600} nm 0.2. After adding the samples, dissolved

299 in 5% of DMSO (0.5MICs = 0.50–5.00 mg/mL for Es and 0.25–8.00 mg/mL for compounds) to
300 5mL of bacteria inoculum dilution, the 24 h incubation at 37 °C ensued. Thereafter, the treated
301 cultures were extracted with chloroform (3 mL), followed by mixing the chloroform layer with
302 0.2 M HCl (1 mL). The absorbance readings (520 nm) of the extracted organic layer were
303 performed using a Shimadzu UV1601 spectrophotometer (Kyoto, Japan).[37] The experiment was
304 done in triplicate and repeated two times. The values were expressed as ratio
305 $(OD_{520}/OD_{600})\times100$.

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

310

311 **RESULTS AND DISCUSSION**

312

313 **Chemical Analysis of *A. pancicii***. *Chemical Composition of EO*. According to obatined
314 results from GC–FID/GC–MS analyses, 81 compound was identified in *A. pacicii* EO (Table 2).
315 Tested oil had the highest percentage of oxygenated sesquiterpenes (34.96%), followed by
316 sesquiterpene hydrocarbons (21.88%) and oxygenated monoterpenes (19.22%). Also, fatty acid
317 derivatives (12.36%), two oxygenated diterpenes (4.41%) and one diterpene hydrocarbon
318 (3.87%) were determined. The alcohols were predominant among oxygenated monoterpenes,
319 sesquiterpenes and fatty acid derivatives (8.88%, 21.32%, 7.91%, respectively). The ester bornyl
320 acetate was the main constituent of *A. pancicii* oil (8.08%) followed by fatty alcohol *n*–octanol
321 (5.82%) and sesquiterpenoids kessane (4.26%) and *β*–selinene (4.26%). Other compounds were

322 present in smaller amounts (<4%) (Table 2). According to literature data, monoterpene
323 hydrocarbons are the most abundant in EOs of many species of this taxa.[38–40] Also, a significant
324 fraction of phtalids can be found in EO of some representatives.[41,42] In this study, the analysis of
325 *A. pancicii* EO showed the highest percentage of sesquiterpenoids (56.84%). In contrast, using
326 GC, GC–MS and HS–GC–MS techniques, Simonović et al.[13] showed that EO obtained from *A.*
327 *pancicii* aerial parts originated from mt. Vidlič (Serbia) possesses high content of
328 monoterpenoids (92.8% and 97.7%, respectively) and notably lower percentage of
329 sesquiterpenoids (4.5% and 1.2%, respectively). As the main constituents they identified *β*–
330 phellandrene, *α*–pinene and *α*–phellandrene, while bornil acetate, which was the most abundant
331 in our study, was recorded in lower percentage (1.3% (GC, GC–MS) and 0.8% (HS–GC–MS)).
332 Also, *δ*–3–karene and mircene were not recorded in the sample from mt. Pelister, while these
333 compounds were present in significant amounts in EO from Vidlič (GC, GC–MS).[13]
334 *Tentative Analysis of MEs*. In order to preliminary identify secondary metabolites of *A.*
335 *pancicii*, MEs of the aerial parts and roots were analysed on a HPLC–DAD/ESI–TOF–MS, in
336 ESI^+ and ESI^- modes. The compounds were structurally assigned in accordance with the exact
337 molecular masses/formulas (Tables 3 and 4). UV data also provided evidence for structure
338 confirmation. The most abundant components of this plant are coumarins, which are
339 characteristic of the genus and could be found in all plant parts.[12]
340 A total of 52 compounds were identified in ME of aerial parts and 53 in ME of the roots
341 (Tables 3 and 4).[13,43–84] Specified structures are mostly in line with literature on *Angelica* species
342 and/or Apiaceae family. Coumarins are represented with 32 compounds found in each analysed
343 plant part: simple coumarins, coumarin glucoside isoscopoletin *β*–D–glucopyranoside,
344 furanocoumarins (including furanocoumarin ethers of falcarindiol–japoangelols C and D and

345 furanocoumarin glycoside nodakenin), pyranocoumarins, and bifuranocoumarins dahuribirin D
346 and rivulobirin A. Besides, this analysis revealed a presence of benzofuran derivatives (*e.g.*
347 glycosidecnidioside A), chromones (*e.g*. noreugeninan 3'–*O*–angeloylhamaudol), chalcones
348 (xanthoangelol J, C and E), sesquiterpenes, polyacetylenes (falcarinone and falcarindiol), fatty
349 acid (pinellic acid) and fatty alcohol (9,12,15–octadecatrien–1–ol), flavonoid glucoside hirsutrin,
350 ester glyceryl linoleate, 3–caffeoyl quinic acid, glycosylated hydroquinone arbutin and lignin
351 kaerophylin (Tables 3 and 4). All identified coumarins have been found previously in the
352 *Angelica* species except bifuranocoumarin rivulobirin A, which was isolated from the roots of
353 *Heracleum rapula* (Apiaceae).[81] Kaerophylin, 3'–*O*–angeloylhamaudol, falcarinone, linolenic
354 and pinelic acid have not been found in the genus *Angelica* so far, but they were found in some
355 species of Apiaceae family. According to literature data, only few angelicas contained
356 chromones *e.g. A. archangelica*,[54] *A. japonica*[65] and *A. polymorpha*.[67]

357 *Isolation and Structure Elucidation*. One new chromone (**1**) and five furanocoumarins
358 (**2**–**6**) were isolated from the crude ME of the roots re–extracted with DCM (plant material
359 collected in 2013). The roots collected in 2014, after a Soxhlet extraction and further separation
360 by semi–preparative reversed phase HPLC, gave furanocumarin **7**, together with compounds **1**,
361 **3**–**5**. According to their NMR, mass spectra, $[\alpha]_D^{22}$ (Supporting Information S2), and a
362 comparison with previously reported spectroscopic data, isolated furanocoumarins were
363 identified as follows: *t*–OMe–oxypeucedanin hydrate (**2**), oxypeucedanin (**3**)[53] (Supporting
364 Information S13–S20), saxalin (**4**), ostruthol (**5**),[67] oxypeucedanin hydrate (**6**) and isoimperatorin
365 (**7**).[48] All of them are linear furanocoumarins with C–5 substitution (Figure 1), derived from
366 isoimperatorin (5–isopentenyloxypsoralene).

367 The new compound **1** was determined as 5'–acetylcnidimol A on the bases of IR,
368 HR–ESI–MS, 1D and 2D NMR spectra (Supporting Information S3–S12) and a comparison with
369 literature which showed that new chromone is derivative of cnidimol A previously found in
370 *Cnidium japonicum*.[85] Cnidimol A is bioactive metabolite which showed high anti-
371 inflammatory activity by significant inhibition of formyl-l-methionyl-l-leucyl-l-
372 phenylalanine/cytochalasin B-induced $O_2^{\bullet-}$generation and/or elastase release.[86] Related
373 compound cnidimoside B is anti-adipogenic chromone which effectively inhibited adipocyte
374 differentiation.[87]

375 The compound **1** was isolated as a white powder. Molecular formula $C_{17}H_{18}O_6$ was
376 deduced from the positive ion mode HR–ESI–MS (*m/z* 319.1184 $[M+H]^+$; calcd. 319.1176 and
377 *m/z* 341.0987 $[M+Na]^+$; calcd. 341.0996). The IR spectrum showed absorption bands
378 characteristic of a hydroxyl (3342 cm^{-1}), an acetyl (1719 cm^{-1}), a pyron carbonyl (1639 cm^{-1})
379 and an aromatic ring (1439 cm^{-1}). In the ^{1}H NMR spectrum (Table 5), a broad singlet of the
380 methyl group at C–2 (C$\underline{H}_3$–11, δ 2.15 *s*), which is coupled with olefinic proton of γ–pyrone ring
381 H–3 (δ 5.81 *br s*), together with singlet at δ 6.16 originated from the aromatic proton H–8,
382 indicated the basic chromone structure of compound **1**, and a 2,5,6,7–substitution. COSY signal
383 C$\underline{H}_3$–11/H–3 confirmed this interpretation. Remaining ^{1}H NMR signals belonged to the
384 substituent at C–6, while signals from OH–5 and OH–7 were missing. 2–Methyl–2–butenyl
385 acetate at C–6 position was deduced from signals of methyl groups OCOC$\underline{H}_3$ and C$\underline{H}_3$–4', an
386 olefinic proton H–2'and methylene protons H_2–1' and H_2–5' (Table 5). The following coupling
387 patterns in the COSY spectrum enabled identification of these protons: C$\underline{H}_3$–4'/H–2', H_2–1', H–
388 2'/ H_2–1'. Carbons were assigned from ^{13}C, DEPT, HSQC and HMBC spectra. DEPT spectrum
389 pointed out two secondary carbons at δ 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 $\underline{C}H_3$–11, $OCO\underline{C}H_3$ and methine carbons C–8, C–3 and C–2'. The lowest field signals at δ 182.3 and 171.9 originated from carbonyls, which were identified as C–4 and $O\underline{C}OCH_3$, respectively, on the basis of HMBC correlations C–4/H–3 and H_2–5', $OCOC\underline{H}_3$/ $O\underline{C}OCH_3$. 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 $C\underline{H}_3$–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).

412 Literature data showed that EOs and Es of various plant parts of *Angelica* species
413 possessed antioxidant activity in a concentration–dependent manner, which is in accordance with
414 results obtained in this work. Similarly to *A. pancicii* Es, *A. koreana* EO and its main
415 components showed significant dose–dependent scavenging activity in DPPH test.[88] For
416 coumarins oxypeucedanin and oxypeucedanin hydrate which were isolated from *A. pancicii* in
417 this study, previously was proven to possess modest antioxidant and cytotoxic activity.[89]
418 *Antibacterial Activity of Es and Isolated Compounds*. The results obtained for *A. pancicii*
419 antibacterial activity (Table 7) revealed that tested samples expressed strong to moderate
420 inhibitory effect on used bacteria. Es obtained from the roots showed the strongest activity
421 among all tested samples, while isolated compounds manifested the minimum inhibition
422 capacity. According to obtained results, EE and ME of the roots had MBCs values in range with
423 those of streptomycin (MBCs = 0.25–5.00 mg/mL). Oxypeucedanin hydrate was the strongest
424 antibacterial agent among compounds, killing all bacteria in the range of MBCs = 0.50–8.00
425 mg/mL, followed by *tert–O*–methyl oxypeucedanin hydrate. The most sensitive bacteria were *B.*
426 *cereus* and *S. aureus*, while the most resistant strains were *L. monocytogenes*, *P. aeruginosa*
427 (Es), *E. coli* and *E. cloacae* (compounds). ME of *A. pancicii* aerial parts showed moderate
428 activity in our research and given results are comparable to previously examined ME of *A. lucida*
429 fruits and its constituents isoimperatorin and oxypeucedanin hydrate, which were also found in
430 ME of *A. pancicii*. Besides, Gram positive and negative bacteria displayed similar sensitivity to
431 both – *A. pancicii* and *A. lucida* species and *P. aeruginosa* appeared to be one of the most
432 resistant strains in both studies.[90]
433 *Antifungal Activity of Es*. Tested fungi showed higher resistance to investigated samples
434 compared to the bacteria. Obtained results for antifungal activity (Table 8) indicated moderate to

435 low potential of Es. EE of roots had the strongest antifungal effect (MFC = 8.00–12.00 mg/mL).
436 *P. funiculosum* was the most sensitive fungus (MFCs = 8.00−18.00 mg/mL), followed by *C.*
437 *albicans* and *T. viride*, while the most resistant were *A. ochraceus* and *A. niger* (MFCs =
438 12.00–>18.00 mg/mL for both strains). *A. pancicii* samples expressed modest activity against *C.*
439 *albicans*, while Es and isolated compounds of *A. lucida* were inactive against assayed *Candida*
440 *s*pecies.[90] The *Aspergillus* fungi were less sensitive to *A. pancicii* Es and this is in agreement
441 with the observations of Roh and Shin,[88] who found that this strain was less vulnerable to
442 activity of *A. koreana* EO. Coumarins oxypeucedanin and oxypeucedanin hydrate, which were
443 detected in *A. pancicii* MEs, manifested good antimicrobial activity in previous study of Stavri
444 and Gibbons[91] and Razavi and Zarrini.[92]

445 *Anti–QS Activity of Es and Isolated Compounds*. All tested samples showed inhibitory
446 effect against *P. aeruginosa* with MICs values in the range of 1.00–5.00 mg/mL for Es and 1.00–
447 16 mg/mL for compounds, which led us to further anti–QS examination of the samples on
448 selected *P. aeruginosa* PAO1 determinants. According to the results given in Table 9, all tested
449 substances were effective in the presence of 0.5MIC. Considering all tested amounts, Es were
450 more effective in comparison to isolated metabolites. In the presence of Es biofilm synthesis of
451 *P. aeruginosa* occurred in lower range (17.36% – 74.53%) than in the presence of ampicillin and
452 streptomycin (49.40% – 92.16%). The highest inhibition activity was observed for EE of the
453 roots (inhibition of 82.64%). Isolated coumarins and new chromone were active in the range of
454 2.72% (0.125MIC of oxipeucedanin hydrate) to 71.60% (0.5MIC of isoimperatorin). The most
455 promising anti–biofilm agents among compounds were osthrutol and oxypeucedanin hydrate.

456 The observation of *P. aeruginosa* twitching and flagella motility and colony formation
457 indicated white to green coloration and modified diameters of treated colonies (Table 9). The

458 most of the colony edges had regular or tiny flagella with size from 16 to 160 μm except colony
459 with acetyl cnidimol A (280 μm). The maximum reduction in diameter was observed in the
460 presence of oxypeucedanin (8.66 mm), followed by EE of roots (9.33 mm) and AE of roots
461 (11.00 mm), and no flagella were noticed. Reduced protrusions were noticed after application of
462 oxypeucedanin, oxypeucedanin hydrate and all Es except AE of aerial parts (Figure 2).

463 The production of pyocyanin was reduced by all *A. pancicii* samples. Tested Es (0.5MIC)
464 demonstrated inhibitory activity against the production of this green pigment (77.49% –
465 114.59%) when compared to the control *P. aeruginosa* (141.55%) (Figure 3). EE of aerial parts
466 showed better inhibition in comparison with streptomycin (84.27%), while AE of roots exhibited
467 better anti–pyocyanin effect (84.68%) than ampicillin (97.56%). The most effective anti–
468 pyocianin agent among isolated compounds was tert–*O*–methyl oxypeucedanin hydrate, enabling
469 91.74% of pyocianin production in contrast to saxalin which allowed 133.54% of its synthesis.
470 New chromone, acetyl cnidimol A, interfered pyocianin production in similar scale as ampicillin
471 (93.72% and 97.56%, respectively) (Figure 3).

472 Earlier reports revealed anti–QS activity of some species from *Angelica* genus. Previous
473 results obtained for anti–QS activity of *A. dahurica* roots ME pointed out this sample as one of
474 the most effective among 97 tested methanol plant Es against *P. aeruginosa* PAO1.[15] Chong et
475 al.[14] confirmed that Es of *A. dahurica* roots exhibited anti–QS properties on *P. aeruginosa*. Also,
476 *A. sinensis* exhibited anti–QS activity by inhibiting selected virulence determinants of the *P.*
477 *aeruginosa* PAO1.[16] Considering all results of anti–QS tests in this work, Es of the roots had
478 moderate potential against QS of PAO1, similar to acetone–aqueous extract of *A. sinensis*
479 roots.[16] MEs of *A. pancicii* with coumarins as the main constituents, exhibited strong anti–
480 biofilm activity. It was proven that furanocoumarins hinder the formation of biofilm in *P.*

481 *aeruginosa*, *E. coli* and *S. typhimurium*[93], indicating the main role of this compounds in strong
482 anti–biofilm activity of *A. pancicii* MEs.

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

492

493 **ASSOCIATED CONTENT**

494

495 ***S Supporting Information**

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

497

498 **AUTHOR INFORMATION**

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509 **Funding**

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

512

513 **Notes**

514 The authors declare no competing financial interest.

515

516 **Abbreviations Used**

517 ABTS, 2,2'–azino–bis(3–ethylbenzothiazoline–6–sulphonic acid); ACN, acetonitrile; AE,
518 aqueous extract; BCB, *β*–carotene bleaching; BHA, 3–tert–butyl–4–hydroxyanisole; CFU,
519 colony forming units; DCM, dichloromethane; DMSO, dimethylsulfoxide; DPPH, 2,2–
520 dyphenyl–1–picrylhydrazyl; EE, ethanol extract; EO, essential oil; Es, extrats; ESI, electrospray
521 ionization interface; GC–FID, gas chromatography–flame ionization detector; GC–MS, gas
522 chromatography–mass spectrometry; HPLC–DAD/ESI–ToF–MS, high pressure liquid
523 chromatography–diode array detector/time–of–flight mass spectroscopy; HS–GC–MS,
524 headspace gas chromatography–mass spectrometry; INT, *p*–iodonitrotetrazolium violet color;
525 LB, Luria–Bertani; MA, Malt agar; MBC, minimum bactericidal concentration; ME, methanol

526 extract; MFC, minimum fungicidal concentration; MH, Mueller–Hinton agar; MIC, minimum
527 inhibitory concentrations; Mt., mountain; OD, optical density; PBS, phosphate buffered saline;
528 QS, quorum sensing; SD, standard deviation; TFC, total flavonoid content; TLC, thin layer
529 chromatography; *t*–OMe–oxypeucedanin hydrate, *tert*–O–methyl–oxypeucedanin hydrate; TPC,
530 total phenolic content; TSB, tryptic soy broth.
531

532 **Author Contributions**

533 K. M. and A. Dž. initiated the research concept. K. M. and S. T. wrote the manuscript. P. M. and
534 V. M. organized plant material collection. V. M. and K. M. collected and prepared plant
535 material. M. R., A. Dž. and K. M. performed GC–MS/GC–FID analysis of essential oil. S. T. and
536 Ž. Š. did the chemical analysis of metanolic extracts, isolated and identified compounds. N. T.
537 conducted the NMR analysis. V. T. guided HPLC–DAD/ESI–ToF–MS analysis. K. M.
538 performed the experiments of biological activity determination. K. M. and A. Ć. prepared and
539 analyzed anti–QS activity of the samples and statistically analyzed the data. A. Dž. and P. M.
540 revised the manuscript.
541

542 **References**

543

544 (1) Nikolić, V. *Angelica* L. In Flora of Republic of Serbia, 5th ed.; Josifivić, M., Ed.; Serbian
545 Academy of Sciences and Arts: Belgrade, Serbia, 1973; pp 268–273.
546 (2) Micevski, K. *Angelica* L. In Flora of Republic of Macedonia, 6th ed.; Matevski, V., Ed.;
547 Macedonian Academy of Sciences and Arts: Skopje, Macedonia, 2005; pp. 1620–1621.

548 (3) Lobiuc, A.; Zamfirache, M. M.; Stratu, A. Physiological aspects in two *Angelica* L. taxa
549 (Apiaceae). *Analele Ştiinţificeale I Universităţii „Al. I. Cuza" Lasis. II a. Biologievegetală* **2012**,
550 *58*, 81–87.
551 (4) Kylin, M. *Angelica archangelica* L. Alnarp: The Faculty of landscape planning horticulture
552 and agriculture science, Swedish University of Agricultural Sciences; 2010.
553 (5) Yu, Y. Processed Chinese angelica (*Angelica sinensis*). *Chinese journal of information on*
554 *traditional Chinese medicine* **2006**, *13*, 45–47.
555 (6) Want, M.; Wu, Q. L.; Simon, J. E.; Jin, Y.; Ho, C. T. Chemistry, pharmacology, and quality
556 control of selected popular Asian herbs in the U.S. market. In Asian Functional Foods; CRC
557 Press: Boca Raton, FL, **2005**; pp. 73–102.
558 (7) Ohnogi, H.; Hayami, S.; Kudo, Y.; Deguchi, S.; Mizutani, S.; Enoki, T.; Tanimura, Y.; Aoi,
559 W.; Naito, Y.; Kato, I.; Yoshikawa, T. *Angelica keiskei* extract improves insulin resistance and
560 hypertriglyceridemia in rats fed a high–fructose drink. *Biosci. Biotechnol. Biochem.* **2012**, *76*,
561 928–932.
562 (8) Chen, C. Y. Elemental analysis of the Taiwanese health food *Angelica keiskei* by INAA. *J.*
563 *Radioanal*. *Nucl*. *Ch*. **2002**, *252*, 551–558.
564 (9) Pervin, M.; Hasnat, M. A.; Debnath, T.; Park, S. M.; Kim, D.; Lim, B. O. Antioxidant, anti–
565 inflammatory and antiproliferative activity of *Angelica dahurica* root extracts. *J. Food Biochem.*
566 **2014**, *38*, 281–292.
567 (10) Vashistha, R. K.; Chaturvedi, A. K.; Nautiyal, B. P.;Nautiyal, M. C. Vegetative propagation
568 of *Angelica glauca* Edgew. and *Angelica archangelica* Linn.: two high value medicinal and
569 aromatic herbs of the Himalaya. *Nat*. *Sci*. **2009**, *7*, 76–82.

570 (11) Hoult, J. R. S.; Payá, M. Pharmacological and biochemical actions of simple coumarins:
571 Natural products with therapeutic potential. *Gen. Pharmacol.* **1996**, *27*, 713–722.
572 (12) Sarker, S. D.; Nahar, L. Natural medicine: the genus *Angelica*. *Curr. Med. Chem.* **2004**, *11*,
573 1479–500.
574 (13) Simonović, S. R.; Stankov–Jovanović, V. P.; Mitić, V. D.; Ilić, M. D.; Petrović, G. M.;
575 Stojanović, G. S. Chemical composition of *Angelica pancicii* essential oil determined by liquid
576 and headspace GC–MS techniques. *Nat. Prod. Commun.* **2014**, *9*, 271–272.
577 (14) Chong, Y. M.; Yin, W. F.; Chan, K. G. The discovery of *Angelica dahurica* roots extracts
578 exhibiting anti–quorum sensing properties. *Planta Med.* 2013, *79*–PL34.
579 (15) Damte, D.; Gebru, E.; Lee, S. J.; Suh, J. W.; Park, S. C. Evaluation of anti–quorum sensing
580 activity of 97 indigenous plant extracts from Korea through bioreporter bacterial strains
581 *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. *J. Microb. Biochem. Technol*. **2013**,
582 *5*, 042–046.
583 (16) Yeo, S. S. M.; Tham, F. Y. Anti–quorum sensing and antimicrobial activities of some
584 traditional Chinese medicinal plants commonly used in South–East Asia. *Malays. J. Microbiol.*
585 **2012**, *8*, 11–20.
586 (17) Rudrappa, T.; Bais, P. H.; Curcumin, a known phenolic from *Curcuma longa*, attenuates the
587 virulence of *Pseudomonas aeruginosa* PAO1 in whole plant and animal pathogenicity models. *J.*
588 *Agric. Food Chem.* **2008**, *56*, 1955–1962.
589 (18) European pharmacopoeia, 7th ed.; Council of Europe, Strasbourg; 2010.
590 (19) Džamić, A.; Soković, M. D.; Novaković, M.; Jadranin, M,; Ristić, M.; Tešević, V.; Marin,
591 P. Composition, antifungal and antioxidant properties of *Hyssopus officinalis* L. subsp. *pilifer*
592 (Pant.) Murb. essential oil and deodorized extracts. *Ind. Crop. Prod.* **2013**, *51*, 401–407.

593 (20) Matos, M. J.; Santana, L.; Uriarte, E.; Abreu, O. A.; Molina, E.; Yordi, E. G. Coumarins –
594 an important class of phytochemicals. In Phytochemicals – isolation, characterisation and role in
595 human health, agricultural and biological sciences. Rao, A. V.; Rao, L. G., Ed.; InTech: 2015;
596 pp. 127–128.
597 (21) Razavi, S. M.; Nahar, L.; Talischi, H.; Sarker, S. D. Ferulone A and ferulone B: two new
598 coumarin esters from *Ferula orientalis* L. roots. *Nat. Prod. Res.* **2016**, *30*, 2183–2189.
599 (22) Adams, R.P. Identification of essential oil components by gas chromatography/mass
600 spectrometry; Allured Publishing Corp.: Carol Stream, IL, USA, 2007.
601 (23) Ivanović, J.; Đilas, S.; Jadranin, M.; Vajs, V.; Babović, N.; Petrović, S.; Žižović, I.
602 Supercritical carbon dioxide extraction of antioxidants from Rosemary (*Rosmarinus Officinalis*
603 L.) and sage (*Salvia Officinalis* L.). *J. Serb. Chem. Soc.* **2009**, *74*, 717–732.
604 (24) Blois, M. S. Antioxidant determinations by the use of a stable free radical. *Nature* **1958**,
605 *181*, 1199–1200.
606 (25) Miller, H. M. A simplified method for the evaluation of antioxidants. *J. Am. Oil Chem. Soc*.
607 **1971**, *45*, 91.
608 (26) Miller, N.; Rice–Evans, C. Factors influencing the antioxidant activity determined by the
609 ABTS radical action assay. *Free Radical Res.* **1997**, *26*, 195–199.
610 (27) Park,Y. K.; Koo, M. H.; Ikegaki, M.; Contado, J. L. Comparison of the flavonoid aglycone
611 contents of *Apis mellifera* propolis from various regions of Brazil. *Braz. Arch. Biol. Techn*. **1997**,
612 *40*, 97–106.
613 (28) Singleton, V. L.; Orthofer, R.; Lamuela–Raventos, R. M. Analysis of total phenols and other
614 oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Method. Enzymol.*
615 **1999**, *299*, 152–178.

616 (29) Booth, C. Fungal culture media. In Methods in microbiology, 4th ed.; Norris, J. R.; Ribbons,
617 D. W., Eds.; Academic Press:London, UK, 1971.
618 (30) Clinical and laboratory standards institute methods for dilution antimicrobial susceptibility
619 test for bacteria that grow aerobically. Aproved Standards, 8th ed.; CLSI publication: Wayne,
620 USA; 2009; M07–A8.
621 (31) Hanel, H.; Raether, W. A. more sophisticated method of determining the fungicidal effect of
622 water–insoluble preparations with a cell harvester, using miconazole as an example. *Mycoses*,
623 **1998**, *31*, 148–154.
624 (32) Tsukatani, T.; Suenaga, H.; Shiga, M.; Noguchi, K.; Ishiyama, M.; Ezoe, T.;Matsumoto, K.
625 Comparison of the WST–8 colorimetric method and the CLSI broth microdilution method for
626 susceptibility testing against drug–resistant bacteria. *J. Microbiol. Meth.* **2012**, *90*, 160–166.
627 (33) Drenkard, E.; Ausubel, F. M. *Pseudomonas* biofilm formation and antibiotic resistance are
628 linked to phenotypic variation. *Nature* **2002**, *416*, 740–743.
629 (34) Spoering, A. L.;Lewis, K. Biofilms and planktonic cells of *Pseudomonas aeruginosa* have
630 similar resistance to killing of antimicrobials. *J. Bacteriol*. **2001**, *183*, 6746–6751.
631 (35) O'Toole, G.A.; Kolter, R. Initiation of biofilm formation in *Pseudomonas fluorescens*
632 WCS365 proceeds *via* multiple convergent signalling pathways: a genetic analysis. *Mol.*
633 *Microbiol.* **1998a**, *28*, 449–461.
634 (36) O'Toole, G. A.; Kolter, R. Flagellar and twitching motility are necessary for *Pseudomonas*
635 *aeruginosa* biofilm development. *Mol. Microbiol.* **1998b**, *30*, 295–304.
636 (37) Sandy, S. M.; Foong–Yee, T. Anti–quorum sensing and antimicrobial activities of some
637 traditional Chinese medicinal plants commonly used in South–East Asia. *Malays. J. Microbiol.*
638 **2012**, *8*, 11–20.

639 (38) Agnihotri, V. K.; Thappa, R. K.; Meena, B.; Kapahi, B. K.; Saxena, R. K.; Qazi, G. N.;
640 Agarwal, S. G. Essential oil composition of aerial parts of *Angelica glauca* growing wild in
641 North–West Himalaya (India). *Phytochemistry* **2004**, *65*, 2411–2413.
642 (39) Doneanu, C.; Anitescu, G. Supercritical carbon dioxide extraction of *Angelica archangelica*
643 L. root oil. *J. Supercrit. Fluid* **1998**, *12*, 59–67.
644 (40) Nivinskiene, O.; Butkiene, R.; Mockute, D. Changes in the chemical compositions of
645 essential oil of *Angelica archangelica* L. roots during storage. *Chemija*, **2003**, *14*, 52–56.
646 (41) Ka, M.–H.; Choi, H. E.; Chun,H.–S.; Lee, K.–G. Antioxidative activity of volatile extracts
647 isolated from *Angelica tenuissimae* roots, peppermint leaves, pine needles, and sweet flag leaves.
648 *J. Agric. Food Chem.* **2005**, *53*, 4124–4129.
649 (42) Wedge, E. D.; Klun, A. J.; Tabanca, N.; Demirci, B.; Ozek, T.; Baser, K. H. C.; Liu, Z.;
650 Zhang, S.; Cantrell, L. C., Zhang, J. Bioactivity-guided fractionation and GC/MS fingerprinting
651 of *Angelica sinensis* and *Angelica archangelica* root components for antifungal and mosquito
652 deterrent activity. *J. Agric. Food Chem.* **2009**, *57*, 464–470.
653 (43) Ge, A. H.; Ma, W. F.; Wang, C. P.; Li, J.; He, J.; Liu, E. W.; Adelakum, T. A.; Zhang, B.
654 L.; Gao, X.; Chang, Y. X. Ultra high performance liquid chromatography with photodiode array
655 detector and quadrupole time–of–flight tandem mass spectrometry coupled with discriminant
656 analysis to evaluate *Angelicae pubescentis radix* from different regions. *J. Sep. Sci.* **2014**, *37*,
657 2523–2534.
658 (44) Kang, S. Y.; Lee, K. Y.; Sung, S. H.; Park, M. J.; Kim, Y. C. Coumarins isolated from
659 *Angelica gigas* inhibit acetylcholine–esterase: structure–activity relationships. *J. Nat. Prod.*
660 **2001**, *64*, 683–685.

661 (45) Kozawa, M.; Baba, K.; Matsuyama, Y.; Hata, K. Studies on coumarins from the root of
662 *Angelica pubescens* Maxim. III. Structures of various coumarins including angelin, a new
663 prenylcoumarin. *Chem. Pharm. Bull.* **1980**, *28*, 1782–1787.
664 (46) Moon, H. I.; Lee, J. H.; Lee, Y. C.; Kim, K. S. Antiplasmodial and cytotoxic activity of
665 coumarin derivatives from dried roots of *Angelica gigas* Nakai *in vitro*. *Immunopharm. Immunot.*
666 **2011**, *33*, 663–666.
667 (47) Liu, J. H.; Xu, S. X.; Yao, X. S.; Kobayashi, H. Two new 6–alkylcoumarins from *Angelica*
668 *pubescens* biserrata. *Planta Med.* **1995b**, *61*, 482–484.
669 (48) Matsuda, H.; Morikawa, T.; Ohgushi, T.; Ishiwada, T.; Nishida, N.; Yoshikawa, M.
670 Inhibitors of nitric oxide production from the flowers of *Angelica furcijuga*: Structures of
671 hyuganosides IV and V. *Chem. Pharm. Bull.* **2005**, *53*, 387–392.
672 (49) Akihisa, T.; Tokuda, H.; Hasegawa, D.; Ukiya, M.; Kimura, Y.; Enjo, F.; Suzuki, T.;
673 Nishino, H. Chalcones and other compounds from the exudates of *Angelica keiskei* and their
674 cancer chemopreventive effects. *J. Nat.Prod.* **2006**, *69*, 38–42.
675 (50) Yang, Y.; Zhang, Y.; Ren, F. X.; Yu, N. J.; Xu, R.;Zhao, Y. M. Chemical constituents in
676 roots of *Angelica polymorpha* Maxim. *Acta Pharmacol. Sin.* **2013**, *48*, 718–722.
677 (51) Wang, B.; Liu, X.; Zhou, A.; Meng, M.; Li, Q. Simultaneous analysis of coumarin
678 derivatives in extracts of *radix Angelicae pubescentis* (Duhuo) by HPLC–DAD–ESI–MSN
679 technique. *Anal. Methods* **2014**, *6*, 7996–8002.
680 (52) Baba, K.; Nakata, K.; Taniguchi, M.; Kido, T.; Kozawa, M. Chemical components of
681 *Angelica keiskei*. Chalcones from *Angelica keiskei*. *Phytochemistry* **1990**, *29*, 3907–3910.

682 (53) Seo, W. D.; Kim, J. Y.; Ryu, H. W.; Kim, J. H.; Han, S. I.; Ra, J. E.; Seo, K. H.; Jang, K. C.;
683 Lee, J. H. Identification and characterisation of coumarins from the roots of *Angelica dahurica*
684 and their inhibitory effects against cholinesterase. *J. Funct. Foods* **2013**, *5*, 1421–1431.
685 (54) Sun, H.; Jakupović, J. Further heraclenol derivatives from *Angelica archangelica.*
686 *Pharmazie* **1986**, *44,* 888–889.
687 (55) An, R. B.; Park, B. Y.; Kim, J. H.; Kwon, O. K.; Lee, J.; Min, B. S.; Ahn, K. S.; Oh, S. R.;
688 Lee, H. K. Coumarins and chromones from *Angelica genuflexa*. *Nat. Prod. Sci.* **2005**, *11*, 79–84.
689 (56) Harkar, S.; Razdan, T. K.; Waight, E. S. Steroids, chromone, and coumarins from *Angelica*
690 *officinalis*. *Phytochemistry* **1984**, *23*, 419–426.
691 (57) Liu, J. H.; Xu, S. X.; Yao, X. S.; Kobayashi, H. Angelol–type coumarins from *Angelica*
692 *pubescens* biserrata, and their inhibitory effect on platelet aggregation. *Phytochemistry* **1995a**,
693 *39*, 1099–1101.
694 (58) Kobayashi, M.; Tawara, T.; Tsuchida, T.; Mitsuhashi, H. Studies on the constituents of
695 Umbelliferae plants. XVIII. Minor constituents of *Bupleuri radix*: occurrence of saikogenins,
696 polyhydroxysterols, a trihydroxy C18 fatty acid, a lignan and a new chromone. *Chem. Pharm.*
697 *Bull.* **1990**, *38*, 3169–3171.
698 (59) Baba, K.; Matsuyama, Y.; Ishida, T.; Inoue, M.; Kozawa, M. Studies on coumarins from the
699 root of *Angelica pubescens* Maxim. V. Stereochemistry of angelols A–H. *Chem. Pharm. Bull.*
700 **1982**, *30*, 2036–2044.
701 (60) Kwon, Y. S.; Shin, S. J.; Kim, M. J.;Kim, C. M. A new coumarin from the stem of *Angelica*
702 *dahurica. Arch. Pharm. Res.* **2002**, *25*, 53–56.

703 (61) Mizuno, A.; Takata, M.; Okada, Y.; Okuyama, T.; Nishino, H.; Nishino, A.; Takayasu, J.
704 Structures of new coumarins and antitumor–promoting activity of coumarins from *Angelica*
705 *edulis*. *Planta Med.* **1994**, *60*, 333–336.
706 (62) Zhao, A.; Yang, X.; Yang, X.; Liu, J.; Wang, Q.; Wang, W. A new natural product from
707 root of *Angelica dahurica* cv. Qibaizhi. *Journal of Chinese Materia Medica* **2012**, *37*,
708 2400–2407.
709 (63) Chi, H. J. Bisabolangelone from *Angelica* spp. *Journal of the Pharmaceutical Society of*
710 *Korea* **1975**, *19*, 115–117.
711 (64) Zheng, L. H.; Wang, P. J.; Li, W.; Feng, D. Chemical composition of essential oil from
712 *Angelica dahurica* roots and its DPPH radical scavenging effect. *Shipin Kexue (Beijing)* **2014**,
713 *35*, 180–183.
714 (65) Kimura, Y.; Sumiyoshi, M.; Taniguchi, M.; Baba, K. Metabolites of 3'–O–acetylhamaudol
715 isolated from *Angelica japonica* roots, and their antitumor actions. *The Natural Products Journal*
716 **2012**, *2*, 20–30.
717 (66) Furumi, K.; Fujioka, T.; Fujii, H.; Okabe, H.; Nakano, Y.; Matsunaga, H.; Katano, M.;
718 Mori, M.; Mihashi, K. Novel antiproliferative falcarindiol furanocoumarin ethers from the root
719 of *Angelica japonica*. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 93–96.
720 (67) Yang, Y.; Yu, N. J.; Liang, F. F.; Zhang, Y.; Ren, F. X.; Zhang, A. J.; Zhao, Y. M. Studies
721 on chemical constituents of *Angelica polymorpha* Maxim (II). *Acta Pharmacol. Sin.* **2010**, *26,*
722 189–191.
723 (68) Avramenko, L. G.; Nikonov, G. K.;Pimenov, M. G. Andelin, a new dihydropyranocoumarin
724 from *Angelica decursiva* roots. *Khim. Prir. Soedin.* **1970**, *6*, 190–194.

725 (69) Baek, N. I.; Ahn, E. M.; Kim, H. Y.; Park, Y. D. Furanocoumarins from the root of *Angelica*
726 *dahurica. Arch. Pharm. Res.* **2000**, *23*, 467–470.
727 (70) Sasaki, H.; Taguchi, H.; Endo, T.; Yosioka, I. The constituents of *Ledebouriella seseloides*
728 Wolff. I. Structures of three new chromones. *Chem. Pharm. Bull.* **1982**, *30*, 3555–3562.
729 (71) Roman, M.; Baranski, R.; Baranska, M. Nondestructive raman analysis of polyacetylenes in
730 Apiaceae vegetables. *J. Agr. Food Chem.* **2011**, *59*, 7647–7653.
731
732 (72) Takahashi, T.; Tsuchida, T.; Uno, T.; Sekita, S.; Satake, M.; Yoshida, N. Study on the
733 botancal origins of "toki" analysis of chemical constituents of wild *Angelica* species distributed
734 in Hokkaido. *J. Nat. Med.* **2005**, *59*, 157–163.
735 (73) Tolibaev, I.; Glushenkova, A. I. Seed lipids of *Heracleum lehmannianum. Khim. Prir. Soed.*
736 **1996**, *1*, 10–14.
737 (74) Mohammadi, M.; Yousefi, M.; Habibi, Z. Essential oils from stem and leaves of *Angelica*
738 *urumiensis* (Mozaffarian) from Iran. *Nat. Prod. Res.* **2010**, *24*, 1347–1351.
739 (75) Chen, Q.; Li, P.; He, J.; Zhang, Z.; Liu, J. Supercritical fluid extraction for identification and
740 determination of volatile metabolites from *Angelica dahurica* by GC–MS. *J. Sep. Sci.* **2008**, *31*,
741 3218–3224.
742 (76) Lee, Y. Y.; Lee, S.; Jin, J. L.; Yun–Choi, H. S. Platelet anti–agregatory effects of coumarins
743 from the roots of *Angelica genuflexa* and *A. gigas. Arc. Pharm. Res.* **2003**, *26*, 723–726.
744 (77) Hua, J. M.; Moon, T. C.; Hong, T. G.; Park, K. M.; Son, J. K.; Chang, H. W. 5–Methoxy–8–
745 (2–hydroxy–3–buthoxy–3–methylbutyloxy)–psoralen isolated from *Angelica dahurica* inhibits
746 cyclooxygenase–2 and 5–lipoxygenase in mouse bone marrow–derived mast cells. *Arc. Pharm.*
747 *Res.* **2008**, *31*, 617–621.

748 (78) Matsuda, H.; Murakami, T.; Kageura, T.; Ninomiya, K.; Toguchida, I.; Nishida, N.;
749 Yoshikawa, M. Hepatoprotective and nitric oxide production inhibitory activities of coumarin
750 and polyacetylene constituents from the roots of *Angelica furcijuga*. *Bioorg. Med. Chem. Lett.*
751 **1998**, *8*, 2191–2196.

752 (79) Gonzalez, J. A.; Estevez–Braun, A.; Estevez–Reyes, R.; Bazzocchi, I. L.; Moujir, L.;
753 Jimenez, I. A.; Ravelo, A.G.; Gonzalez, A. G. Biological activity of secondary metabolites from
754 *Bupleurum salicifolium* (Umbelliferae). *Experientia* **1995**, *51*, 35–39.

755 (80) Wang, N. H.; Yoshizaki, K.; Baba, K. Seven new bifuranocoumarins, dahuribirin A–G,
756 from Japanese BaiZhi. *Chem. Pharm. Bull.* **2001**, *49*, 1085–1088.

757 (81) Niu, X. M.; Li, S. H.; Jiang, B.; Zhao, Q. S.; Sun, H. D. Constituents from the roots of
758 *Heracleum rapula* Franch. *J. Asian Nat. Prod. Res.* **2002**, *4*, 33–41.

759 (82) Song, P.; Sun, M.; Xu, Z.; Wang, N. Chemical constituents in three plants of *Angelica L.*
760 *Chinese traditional and herbal drugs* **2007**, *38*, 833–835.

761 (83) Akihisa, T.; Tokuda, H.; Ukiya, M.; Iizuka, M.; Schneider, S.; Ogasawara, K.; Mukainaka,
762 T.; Iwatsuki, K.; Suzuki, T.; Nishino, H. Chalcones, coumarins, and flavanones from the exudate
763 of *Angelica keiskei* and their chemopreventive effects. *Cancer Lett.* **2003**, *201*, 133–137.

764 (84) Li, L.; Lu, L.; Zhang, W.; Zhao, J.; Lu, D.; Lou, Z. GC–MS combined with PCA analysis of
765 essential oil from two varieties of *radix Angelica edahuricae*. *Chinese journal of pharmaceutical*
766 *analysis* **2011**, *31*, 112–118.

767 (85) Baba, K.; Kawanishi, H.; Taniguchi, M.; Kozawa, M. Chromone glucosides from *Cnidium*
768 *japonicum*. *Phytochemistry* **1993**, *35*, 221–22.

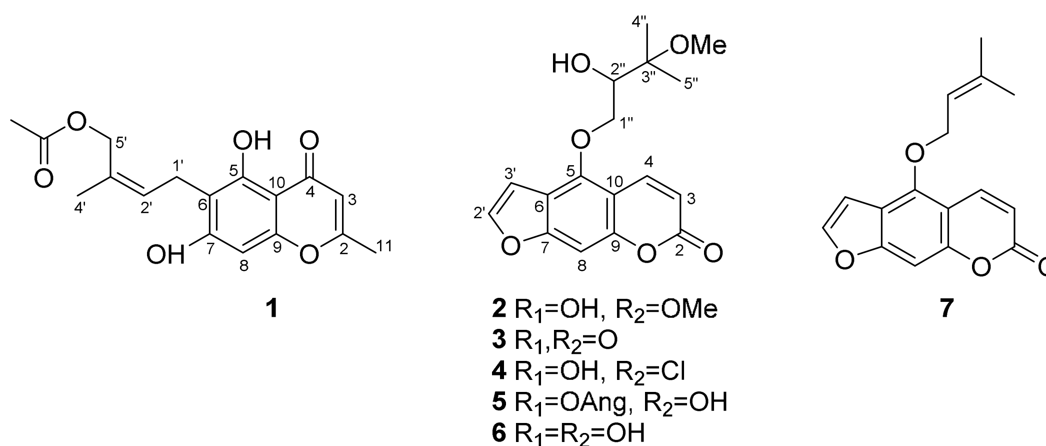
769 (86) Lee, T.–H.; Chen, Y.–C.; Hwang, T.–L.; Shu, C.–W.; Sung, P.–J.; Lim, Y.–P.; Kuo, W.–
770 L.; Chen, J.–J. New coumarins and anti–inflammatory constituents from the fruits of *Cnidium*
771 *monnieri*. *Int. J. Mol. Sci.* **2014**, *15*, 9566–9578
772 (87) Seon B. K.; Jong H. A.; Sang–Bae, H.; Bang Y. H.; Sung Y. K.; Mi K. L. Anti-
773 adipogenic chromone glycosides from *Cnidium monnieri* fruits in 3T3-L1 cells. *Bioorg. Med.*
774 *Chem. Lett.* **2012**, *22*, 6267–6271.
775 (88) Roh, J.; Shin, S. Antifungal and antioxidant activities of the essential oil from *Angelica*
776 *koreana* Nakai. *Evid. Based Complement. Alternat. Med*. **2014**, *2014*, 7 pages.
777 (89) Zahri,S.; Razavi, S. M.; Moatamed, Z. Antioxidant activity and cytotoxic effect of aviprin
778 and aviprin–3″–*O*–D–glucopyranoside on LNCaP and HeLa cell lines. *Nat. Prod. Res.* **2012**, *26*,
779 540–547.
780 (90) Widelski, J.; Popova, M.; Graikou, K.; Glowniak, K.;Chinou, I. Coumarins from *Angelica*
781 *lucida* L.–antibacterial activities. *Molecules* **2009**, *14*, 2729–2734.
782 (91) Stavri, M.; Gibbons, S. The antimycobacterial constituents of dill (*Anethum graveolens*).
783 *Phytother. Res.* **2005**, *19*, 938–941.
784 (92) Razavi, S. M.; Zarrini, G. Bioactivity of aviprin and aviprin–3"–*O*–glucoside, two linear
785 furanocoumarins from Apiaceae. *Russ. J. Bioorg. Chem.* **2010**, *36*, 359–362.
786 (93) Heggers, J. P.; Cottingham, J.; Gusman, J.; Reagor, L.; McCoy, L.; Carino, E.; Cox, R.;
787 Zhao, J. G. The effectiveness of processed grapefruit–seed extract as an antibacterial agent: II.
788 Mechanism of action and *in vitro* toxicity. *J. Altern. Complement. Med.* **2002**, *8*, 333–340.
789
790
791

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

793

	A. pancicii Es					
	Methanol		Ethanol		Aqueous	
Amount 10 g	Aerial parts	Roots	Aerial parts	Roots	Aerial parts	Roots
Yield (g)	1.236	1.905	0.654	1.236	0.987	1.543

794



795

796 **Figure 1.** The structures of isolated compounds **1**–**7**.

797

798 **Table 2. Chemical composition of EO ofA. *pancicii* aerial parts**

799

Compounds	**KIE**	**KIL**	**%**
n.i.*	–	–	0.17
n–Heptanal	900.6	901	0.20
α–Pinene	932.2	932	0.66
4–Methylopent–2–enolide	947.8	945	0.13
n–Heptanol	959.1	959	0.05

3–*p*–Menthene	985.7	984	0.06
2–Penthylfuran	986.0	993	0.13
n–Octanal	995.0	998	1.26
α–Terpinene	1016.4	1014	0.08
p–Cymene	1019.8	1020	0.56
β–Phellandrene	1023.2	1025	0.55
Benzene acetaldehyde	1041.2	1036	0.17
***n*–Octanol**	**1068.5**	**1063**	**5.82**
m–Cymenene	1082.9	1083	0.58
2–Nonanone	1087.3	1087	0.08
n–Nonanal	1099.1	1100	0.51
trans–Sabinene hydrate (IPP vs OH)	1112.1	1098	3.32
Octyl formate	1125.6	1127	0.38
trans–*p*–Menth–2–en–1–ol	1133.5	1136	3.26
trans–Sabinol (*trans* for OH vs. IPP)	1143.8	1137	0.59
Borneol	1164.9	1165	0.35
p–Cymen–8–ol	1178.0	1179	0.24
cis–Piperitol	1192.9	1195	0.94
Verbenone	1204.1	1204	1.43
Octanol acetate	1209.7	1211	2.21
trans–Carveol	1219.7	1215	0.18
Piperitone	1252.2	1249	0.47
2E–Decenal	1263.6	1260	0.40
Bornyl acetate	**1287.0**	**1287**	**8.08**
Lavandulyl acetate	1291.4	1288	0.38
α–Longipinene	1349.4	1350	0.43
α–Ylangene	1373.0	1373	0.28
Isoledene	1375.5	1374	0.45

α–Copaene	1378.3	1374	0.82
Daucene	1382.3	1380	0.57
β–Bourbonene	1387.2	1387	0.50
β–Elemene	1385.7	1389	2.73
β–Funebrene	1415.2	1413	0.45
β–Cedrene	1421.6	1419	0.60
trans–*α*–Bergamotene	1438.2	1432	0.39
α–Himachalene	1451.3	1449	0.27
7–*epi*–1,2–Dehydro–sesquicineole	1474.5	1471	0.83
α–Amorphene	1479.7	1483	1.70
cis–Eudesma–6,11–diene	1483.5	1489	1.17
***β*–Selinene**	**1489.2**	**1489**	**4.25**
β–Dihydroagarofuran	1490.4	1496	0.98
α–Selinene	1495.2	1498	1.79
Isodaucene	1496.9	1500	1.00
β–Bisabolene	1505.0	1505	0.43
Isobornyl isovalerate	1513.3	1521	1.06
Kessane	**1524.1**	**1529**	**4.26**
Selina–3,7(11) –diene	1532.9	1545	0.32
α–Calacorene	1538.2	1544	0.83
Elemol	1548.0	1548	0.58
cis–Muurol–5–en–4–α–ol	1551.1	1559	0.31
β–Calacorene	1559.1	1559	0.43
E–Nerolidol	1562.3	1561	1.25
Spathulenol	1579.4	1577	2.58
Globulol	1584.8	1590	2.26
Viridiflorol	1594.9	1593	0.81
Humulene epoxide II	**1607.9**	**1608**	**3.51**

1–*epi*–Cubenol	1626.9	1627	0.70
β–Acorenol	1632.5	1636	2.16
Cadalene	1671.1	1675	1.40
α–Bisabolol	1684.9	1685	2.46
Germacra–4(15),5,10(14) –trien–1–*α*–ol	1691.7	1685	0.55
Acorenone B	1700.5	1697	0.46
Amorpha–4,9–dien–2–ol	1702.2	1700	1.70
Nootkatol	1718.3	1714	1.42
Z–9–Pentadecenol**	1723.2	n.i.	1.64
Z–*α*–Atlantone	1718.5	1717	1.11
izo–Longifolol	1722.2	1728	1.16
Eremophilon	1731.7	1734	1.15
Cedr–8(15) –en –9–α–ol acetate	1747.6	1741	0.59
epi–Cyclocolorenone	1772.7	1774	0.88
Acorone	1816.2	1819	0.70
Neophytadiene	**1835.1**	**1835**	**3.87**
α–Chenopodiol	1853.5	1855	1.62
Flourensadiol	1873.5	1869	0.91
3–(4,8,12–Trimethyltridecyl) furan**	1962.0	n.i.	2.67
Phytol	2103.0	2103	1.74
Class			
Monoterpene hydrocarbons			**2.49**
Oxygenated monoterpenes			**19.22**
Sesquiterpene hydrocarbons			**21.88**
Oxygenated sesquiterpenes			**34.96**
Fatty acidsderivates			**12.36**
Diterpenehydrocarbons			**3.87**
Oxygenated diterpenes			**4.41**

Others	**0.80**
Total	**99.83**

800 KIE–Kovats (retention) index experimentally determined (AMDIS)

801 KIL–Kovats (retention) index – literature data (Adams, 2007)

802 * n.i.– not identified

803 **– tentatively assigned

804

805 **Table 3. Preliminary LC–MS analysis of chemical composition of *A. pancicii* aerial parts**

806 **ME**

807

Rt (min) ESI$^+$	**Rt (min)** ESI$^-$	**TOFMS (*m/z*) [ESI$^+$/ ESI$^-$]**	**Formula**	**Compound**
5.382	5.364	355.1026 $[M + H]^+$ 353.0890 $[M – H]^–$,	$C_{16}H_{18}O_9$	Chlorogenic acid[43]
5.382		163.0389 $[M + H]^+$	$C_9H_6O_3$	Umbelliferone[44]
	5.843	287.0048 $[M – H]^–$,	$C_{16}H_{16}O_5$	Angelicone[45] Columbianetin acetate[43]
	6.464	399.0943 $[M+HCO_2]^–$ 389.0656 $[M+Cl]^–$	$C_{16}H_{18}O_9$	Isoscopoletin β–D–glucopyranoside[46]
6.767		295.1175 $[M + H]^+$	$C_{15}H_{18}O_6$	Angelitriol[47]
6.767	6.766	369.1178 $[M + H]^+$ 367.1043 $[M – H]^–$	$C_{17}H_{20}O_9$	Cnidioside A[45]
	6.766	455.1572 $[M + HCO_2]^–$	$C_{25}H_{30}O_5$	Xanthoangelol J[49]
7.323		465.1027 $[M + H]^+$	$C_{21}H_{20}O_{12}$	Hirsutrin[45]
7.601	7.831	193.0495 $[M + H]^+$ 191.0355 $[M – H]^–$	$C_{10}H_8O_4$	Noreugenin[47]

	7.636	271.0359 [M – H]$^-$	$C_{12}H_{16}O_7$	Arbutin[45]
8.543		197.1172 [M + H]$^+$	$C_{11}H_6O_3$	Psoralen[51]
9.802		193.0499 [M + H]$^+$	$C_{10}H_8O_4$	Scopoletin[52]
	9.800	191.0354 [M – H]$^-$		
10.636		305.1019 [M + H]$^+$ 631.1782 [2M + Na]$^+$	$C_{16}H_{16}O_6$	Oxypeucedanin hydrate[53] Heraclenol[54]
	11.683	275.0935 [M – H]$^-$	$C_{15}H_{16}O_5$	Hamaudol[55]
11.754		203.0341 [M + H]$^+$	$C_{11}H_6O_4$	Xanthotoxol, Bergaptol[56]
12.234		377.1592 [M + H]$^+$ 399.1414 [M + Na]$^+$	$C_{20}H_{24}O_7$	Angelol A[45], G[57] H[53], B, D, K[57]
	12.623	329.2345 [M – H]$^-$ 421.1512 [M+HCO$_2$]$^-$	$C_{18}H_{34}O_5$	Pinellic acid[58]
12.801		377.1589 [M + H]$^+$ 399.1414 [M + Na]$^+$ 775,2927 [2M + Na]$^+$	$C_{20}H_{24}O_7$	Angelol A[45], G[57] H[53], B, D, K[57]
12.997		379.1751 [M + H]$^+$ 779.3240 [2M + Na]$^+$	$C_{20}H_{26}O_7$	Angelol C, E, F[59], L[51] I[60]
	12.978	423.1673 [M+HCO$_2$]$^-$ 413.1381 [M+ Cl]$^-$		
12.997		361.1644 [M + H]$^+$	$C_{19}H_{20}O_7$	Edulisin IV[61]
13.280		377.1591 [M + H]$^+$ 399.1414 [M + Na]$^+$ 775.2927 [2M + Na]$^+$	$C_{20}H_{24}O_7$	Angelol A[45], G[57] H[53], B, D, K[57]
	13.280	421.1517 [M+HCO$_2$]$^-$ 411.1228 [M+ Cl]$^-$		
13.511		379.1758 [M + H]$^+$	$C_{20}H_{26}O_7$	Angelol C, E, F[59], L[51], I[60]
	13.493	423.1675 [M+HCO$_2$]$^-$		

		413.1378 [M+ Cl]$^-$		
13.511		219.0649 [M + H]$^+$	$C_{12}H_{10}O_4$	6–Acetyl–7–methoxycoumarin[62]
14.683		231.1367 [M + H]$^+$	$C_{15}H_{18}O_2$	4–Benzofuranol, 3,6–dimethyl–2–(3–methyl–2–buten–1–yl)[63]
		249.1484 [M + H]$^+$ 519.2717 [2M + Na]$^+$	$C_{15}H_{20}O_3$	Bisabolangelone[63]
14.705		149.0975 [M + H]$^+$	$C_{10}H_{12}O$	Estragole[64]
15.091	15.090	319.1174 [M + H]$^+$ 341.0993 [M + Na]$^+$ 317.1178 [M – H]$^-$	$C_{17}H_{18}O_6$	Acetyl cnidimol A, *t*–OMe–oxypeucedanin hydrate[53], 3'–O–Acetylhamaudol[65]
	15.712	367.0601 [M+HCO$_2$]$^-$ 357.0314 [M+ Cl]$^-$	$C_{20}H_{24}O_7$	Angelol A[45], G[57] H[53], B, D, K[57]
16.564	16.564	261.1121 [M + H]$^+$ 259.0981 [M – H]$^-$	$C_{15}H_{16}O_4$ $C_{17}H_{24}O_2$	7–Methoxy–5–prenyloxy–coumarin[44], Falcarindiol[66]
16.866	16.840	387.1439 [M + H]$^+$ 795.2068 [2M + Na]$^+$ 431.1359 [M+HCO$_2$]$^-$ 421.1072 [M+ Cl]$^-$	$C_{21}H_{22}O_7$	Ostruthol[67], Tomazin[54]
17.895		359.1490 [M + H]$^+$ 739.2719 [2M + Na]$^+$	$C_{20}H_{22}O_6$	7,8–Dihydro–7–hydroxy–6–methoxy–8,8–dimethyl–2H,6H–benzo[1,2–b:5,4–b']dipyran–2–one–crotonic acid–3–methyl–ester[68]
18.108		361.1646 [M + H]$^+$ 743.3029 [2M + Na]$^+$	$C_{20}H_{24}O_6$	Oxypeucedanin hydrate–3''–tert–butyl ether[69]
		259.0966 [M + H]$^+$	$C_{15}H_{14}O_4$	7–Methoxy–8–senecioyl coumarin[45], Pablohopin[62]
	18.767	357.1354 [M – H]$^-$	$C_{20}H_{22}O_6$	3'–O–Angeloylhamaudol[70]

ESI+	ESI−	TOFMS (m/z)	Formula	Compound
19.883		243.1745 $[M + H]^+$	$C_{17}H_{22}O$	Falcarinone[71]
	20.052	295.2287 $[M – H]^−$ 331.2055 $[M+ Cl]^−$	$C_{18}H_{32}O_3$	13–Hydroxy–9(Z), 11(E) –octadecadienoic acid, 9–Hydroxy–10(E), 12(Z) –octadecadienoic acid [72]
20.078		279.2318 $[M + H]^+$	$C_{18}H_{30}O_2$	Linolenic acid[73]
23.664		280.2637 $[M + NH_4]^+$	$C_{18}H_{30}O$	*E,E*–Farnesylacetone[74]
24.391		355.2839 $[M + H]^+$ 377.2659 $[M + Na]^+$	$C_{21}H_{38}O_4$	1–Glyceryl linoleate[75]

*Mass accuracy within 5 ppm

Table 4. Preliminary LC–MS analysis of chemical composition of *A. pancicii* roots ME

Rt (min)		**TOFMS (*m/z*)**	**Formula**	**Compound**
ESI$^+$	**ESI$^−$**	**[ESI$^+$/ ESI$^−$]**		
5.375	5.370	355.1024 $[M + H]^+$ 353.0892 $[M – H]^−$	$C_{16}H_{18}O_9$	Chlorogenic acid[43]
5.375		163.0391 $[M + H]^+$	$C_9H_6O_3$	Umbelliferone[44]
	6.748	367.1042 $[M – H]^−$	$C_{17}H_{20}O_9$	Cnidioside A[48]
	7.970	453.1141 $[M + HCO_2]^−$ 443.1145$[M + Cl]^−$	$C_{20}H_{24}O_9$	Nodakenin[44]
	8.251	515.1203 $[M – H]^−$	$C_{25}H_{24}O_{12}$	Isochlorogenic acid A[43]
	8.749	365.0440 $[M – H]^−$	$C_{23}H_{10}O_5$	Xanthoangelol C[52]
10.609	10.613	305.1020 $[M + H]^+$ 349.0953 $[M + HCO_2]^−$ 339.0657 $[M + Cl]^−$	$C_{16}H_{16}O_6$	Oxypeucedanin hydrate[53], Heraclenol[54]
	11.678	275.0936 $[M – H]^−$	$C_{15}H_{16}O_5$	Hamaudol[55]

12.573	12.630	277.1078 $[M + H]^+$ 329.2356 $[M – H]^–$	$C_{18}H_{34}O_5$	Pinellic acid[58]
13.026	13.022	379.1761$[M + H]^+$ 423.1669$[M + HCO_2]^–$ 413.1389 $[M + Cl]^–$	$C_{20}H_{26}O_7$	Angelol C, E, F[59], L[51], I[60]
13.026		361.1645 $[M + H]^+$	$C_{19}H_{20}O_7$	Edulisin IV[61]
13.495		379.1750$[M + H]^+$	$C_{20}H_{26}O_7$	Angelol C, E, F[59], L[51], I[60]
13.752	13.719	319.1297 $[M + H]^+$ 363.1097 $[M + HCO_2]^–$ 353.0811 $[M + Cl]^–$	$C_{17}H_{18}O_6$	Acetyl cnidimol A, *t*–OMe–oxypeucedanin hydrate[53], 3'–O–Acetylhamaudol[65]
13.752		287.0878 $[M + H]^+$	$C_{16}H_{14}O_5$	Heraclenin[55], Isooxypeucedanin[76], Oxypeucedanin[53]
14.699		249.1484 $[M + H]^+$	$C_{15}H_{20}O_3$	Bisabolangelone[63]
15.046		287.0911 $[M + H]^+$	$C_{16}H_{14}O_5$	Pabulenol[76]
15.087	15.086	319.1176 $[M + H]^+$ 317.1083 $[M – H]^–$	$C_{17}H_{18}O_6$	Acetyl cnidimol A, *t*–OMe–oxypeucedanin hydrate[53], 3'–O–Acetylhamaudol[65]
	15.576	229.0875 $[M – H]^–$	$C_{14}H_{14}O_3$	Osthenol[51]
15.718		323.0686 $[M+H+2]^+$	$C_{16}H_{15}ClO_5$	Saxaline[66]
	16.561	259.0999 $[M – H]^–$	$C_{15}H_{16}O_4$	7–Methoxy–5–prenyloxy–coumarin[44]
	16.826	385.1313 $[M – H]^–$	$C_{21}H_{22}O_7$	Ostruthol[66], Tomazin[54]
16.830		391.1750 $[M + H – ACN]^+$	$C_{21}H_{26}O_7$	5–methoxy–8– (2–hydroxy–3–buthoxy–3–methylbutyloxy) –psoralen[77]
17.300	17.340	389.1594 $[M + H]^+$ 433.1513 $[M + HCO_2]^–$	$C_{21}H_{24}O_7$	Suksdorfin[78]

		423.1225 [M + Cl]$^-$		
	17.813	327.1251 [M – H]$^-$	$C_{19}H_{20}O_5$	Decursin, Decursinol angelate[76], Columbianadin[43]
18.630		271.0965 [M + H]$^+$	$C_{16}H_{14}O_4$	Imperatorin, Isoimperatorin[48]
	19.864	243.1747 [M + H]$^+$ 485.3412 [2M + H]$^+$	$C_{17}H_{22}O$	Falcarinone[71]
20.555		369.1333 [M + H]$^+$	$C_{21}H_{20}O_6$	Kaerophylin[79]
20.975		371.1494 [M + H]$^+$	$C_{21}H_{22}O_6$	Xanthoangelol E[52]
21.472		573.1747 [M + H]$^+$	$C_{32}H_{28}O_{10}$	Dahuribirin D[80]
21.794		573.1747 [M + H]$^+$	$C_{32}H_{28}O_{10}$	Rivulobirin A[81]
22.070		387.1434 [M + H]$^+$	$C_{21}H_{22}O_7$	Peucenidin[82], Isopeucenidin, Edultin, Pteryxin[83], Isopteryxin[78]
22.401		205.1952[M + H]+	$C_{15}H_{24}$	*α*–Humulene, *α*–Funebrene, *β*–Bourbonene[13]
23.642		280.2633 [M+NH$_4$]$^+$	$C_{18}H_{30}O$	*E,E*–Farnesylacetone[74]
	24.315	591.2614 [M + HCO$_2$]$^-$ 581.2321 [M + Cl]$^-$	$C_{33}H_{38}O_7$	Japoangelol C, Japoangelol D[66]
25.428		282.2799 [M+NH$_4$]$^+$	$C_{18}H_{32}O$	9,12,15–Octadecatrien–1–ol[84]

*Mass accuracy within 5 ppm

Table 5. NMR data of compound **1**

Position H/C	δ_C, multiplicity	δ_H, multiplicity (*J* in Hz)	HMBC (H→C)	NOESY (H→H)
2	166.6, qC	/	/	/
3	107.7, CH	5.81 *br s*	2	/
4	182.3, qC	/	/	/

5	156.2, qC	/	/	/
6	110.2, qC	/	/	/
7	161.7, qC	/	/	/
8	93.0, CH	6.16 *s*	6, 7, 9, 10	/
9	156.2, qC	/	/	/
10	103.7, qC	/	/	/
11	19.8, CH_3	2.15 *br s*	2	3
1'	20.7, CH_2	3.22 *d* (7.5)	5, 6, 7	5'
2'	127.8, CH	5.38 *br t* (7.5)	/	4'
3'	129.3, qC	/	/	/
4'	20.9, CH_3	1.54 *d* (0.5)	2', 3', 5'	2', 5'
5'	63.4, CH_2	4.65 *s*	2', 3', O$\underline{C}$OCH$_3$	1', 4'
5'-OCOCH$_3$	20.4, OCO$\underline{C}$H$_3$ 171.9, O$\underline{C}$OCH$_3$	1.91 *s* OCOC$\underline{H}_3$	OCOC$\underline{H}_3$/O$\underline{C}$OCH$_3$	/

816

817 **Table 6. Results of TPCs, TFCs and antioxidant activity of *A. pancicii* Es and standards**

818 **(means ± SD)**

819

Assay/ *A. pancicii* Es/Standards		**Total phenolic contents**		**Antioxidant activity**		
		TPC 1 mg/mL (mg GA/g of DE)	**TFC 1 mg/mL (mg QE/g of DE)**	**DPPH (IC_{50} = mg/mL)**	**ABTS 1 mg/mL (mg Vit. C/g of DE)**	**BCB (IC_{50} = mg/mL)**
ME	**Aerial parts**	72.77 ± 0.00^{c}	35.15 ± 0.00^{a}	0.29 ± 0.01^{b}	0.87 ± 0.00^{c}	2.98 ± 0.02^{b}
	Roots	66.68 ± 0.00^{d}	4.46 ± 0.00^{c}	0.40 ± 0.01^{c}	0.90 ± 0.00^{c}	4.94 ± 0.07^{c}
EE	**Aerial parts**	143.99 ± 0.01^{a}	31.39 ± 0.01^{a}	0.26 ± 0.01^{b}	1.10 ± 0.00^{b}	2.45 ± 0.00^{b}

	Roots	90.33 ± 0.01^{b}	4.00 ± 0.00^{c}	0.47 ± 0.00^{c}	1.06 ± 0.01^{b}	5.54 ± 0.00^{c}
AE	**Aerial parts**	84.81 ± 0.00^{c}	9.54 ± 0.00^{b}	0.28 ± 0.01^{b}	0.98 ± 0.01^{b}	2.54 ± 0.01^{b}
	Roots	54.37 ± 0.00^{d}	6.38 ± 0.01^{c}	0.41 ± 0.01^{c}	0.64 ± 0.00^{d}	14.00 ± 0.01^{d}
Standards		n.d.	n.d.	BHA 0.13 ± 0.01^{a} Vit C 0.03 ± 0.01^{a}	QE 2.75 ± 0.00^{a}	BHA 1.22 ± 0.02^{a}

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

821 n.d. – not determined

822 **Table 7. Results of antibacterial activity of *A. pancicii* Es, isolated compounds and streptomycin in mg/mL (means ± SD)**

823

Bacteria/ *A. pancicii* Es/Standard			Gram–positive bacteria				Gram–negative bacteria			
			B. cereus	*M. flavus*	*L. monocytogenes*	*S. aureus*	*P. aeruginosa*	*E. coli*	*E. cloacae*	*S. tiphymurium*
ME	Aerial parts	**MIC**	0.50 ± 0.02^{a}	4.00 ± 0.06^{c}	2.00 ± 0.03^{b}	1.00 ± 0.03^{a}	2.00 ± 0.11^{b}	4.00 ± 0.02^{c}	1.00 ± 0.04^{a}	1.00 ± 0.01^{a}
		MBC	1.00 ± 0.03^{a}	7.00 ± 0.02^{b}	3.00 ± 0.02^{a}	2.00 ± 0.05^{a}	3.00 ± 0.06^{a}	8.00 ± 0.10^{b}	3.00 ± 0.02^{a}	3.00 ± 0.01^{a}
	Roots	**MIC**	0.20 ± 0.04^{a}	0.25 ± 0.03^{a}	1.00 ± 0.00^{b}	0.20 ± 0.02^{a}	1.00 ± 0.05^{c}	0.50 ± 0.06^{b}	0.25 ± 0.03^{a}	1.00 ± 0.05^{c}
		MBC	0.25 ± 0.03^{a}	0.50± 0.05^{a}	5.00 ± 0.01^{b}	0.25 ± 0.03^{a}	3.00 ± 0.03^{b}	1.00 ± 0.02^{a}	0.50 ± 0.03^{a}	2.00 ± 0.07^{b}
EE	Aerial parts	**MIC**	1.00 ± 0.02^{a}	4.00 ± 0.02^{b}	1.00 ± 0.02^{a}	2.00 ± 0.05^{a}	1.00 ± 0.03^{a}	4.00 ± 0.03^{b}	2.00 ± 0.01^{a}	2.00 ± 0.04^{a}
		MBC	2.00 ± 0.05^{a}	7.00 ± 0.01^{c}	3.00 ± 0.05^{a}	4.00 ± 0.07^{b}	3.00 ± 0.02^{a}	6.00 ± 0.03^{c}	3.00 ± 0.07^{a}	3.00 ± 0.08^{a}
	Roots	**MIC**	0.30 ± 0.03^{a}	0.40 ± 0.02^{a}	2.00 ± 0.07^{b}	0.20 ± 0.01^{a}	1.00 ± 0.03^{b}	0.30 ± 0.04^{a}	0.30 ± 0.07^{a}	0.30 ± 0.04^{a}
		MBC	0.40 ± 0.01^{a}	0.50 ± 0.07^{a}	4.00 ± 0.00^{c}	0.30 ± 0.01^{a}	2.00 ± 0.02^{b}	0.40 ± 0.02^{a}	0.40 ± 0.03^{a}	0.40 ± 0.00^{a}
AE	Aerial parts	**MIC**	5.00 ± 0.09^{a}	10.00 ± 0.03^{b}	5.00 ± 0.07^{a}	10.00 ± 0.08^{b}	10.00 ± 0.05^{b}	10.00 ± 0.06^{b}	10.00 ± 0.07^{b}	10.00 ± 0.03^{b}
		MBC	10.00 ± 0.05^{a}	>14.00 ± 0.02^{b}	11.00 ± 0.11^{a}	14.00 ± 0.05^{b}	11.00 ± 0.04^{a}	11.00 ± 0.08^{a}	>14.00 ± 0.05^{b}	>14.00 ± 0.02^{b}
	Roots	**MIC**	5.00 ± 0.09^{b}	10.00 ± 0.10^{c}	5.00 ± 0.01^{b}	5.00 ± 0.03^{b}	5.00 ± 0.07^{a}	10.00 ± 0.08^{c}	4.00 ± 0.07^{b}	9.00 ± 0.05^{c}
		MBC	6.00 ± 0.02^{a}	>14.00 ± 0.05^{c}	11.00 ± 0.05^{b}	7.00 ± 0.14^{a}	11.00 ± 0.05^{b}	>14.00 ± 0.06^{c}	5.00 ± 0.03^{a}	10.00 ± 0.01^{b}
tert–O–methyl oxypeucedanin hydrate		**MIC**	0.50 ± 0.02^{a}	16.00 ± 0.12^{c}	1.00 ± 0.02^{a}	2.00 ± 0.06^{a}	8.00 ± 0.03^{b}	16.00 ± 0.09^{c}	1.00 ± 0.07^{a}	1.00 ± 0.02^{a}
		MBC	1.00 ± 0.03^{a}	n.i.	8.00 ± 0.05^{b}	8.00 ± 0.02^{b}	16.00 ± 0.05^{c}	n.i.	2.00 ± 0.03^{a}	2.00 ± 0.00^{a}

Oxypeucedanin	**MIC**	2.00 ± 0.03[a]	16.00 ± 0.05[b]	4.00 ± 0.04[a]	4.00 ± 0.01[a]	4.00 ± 0.08[a]	16.00 ± 0.02[b]	16.00 ± 0.07[b]	4.00 ± 0.05[a]
	MBC	4.00 ± 0.06[a]	n.i.	16.00 ± 0.09[c]	16.00 ± 0.05[c]	8.00 ± 0.02[b]	n.i.	n.i.	16.00 ± 0.07[c]
Saxalin	**MIC**	8.00 ± 0.11[b]	16.00 ± 0.03[c]	8.00 ± 0.03[b]	8.00 ± 0.08[b]	8.00 ± 0.05[b]	16.00 ± 0.03[c]	1.00 ± 0.03[a]	1.00 ± 0.03[a]
	MBC	16.00 ± 0.05[b]	n.i.	16.00 ± 0.00[b]	16.00 ± 0.09[b]	16.00 ± 0.04[b]	n.i.	2.00 ± 0.04[a]	2.00 ± 0.02[a]
Ostruthol	**MIC**	16.00 ± 0.03[b]	16.00 ± 0.05[b]	8.00 ± 0.04[a]	8.00 ± 0.07[a]	16.00 ± 0.02[b]	16.00 ± 0.07[b]	16.00 ± 0.00[b]	16.00 ± 0.02[b]
	MBC	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Oxypeucedanin hydrate	**MIC**	n.i.	2.00 ± 0.00[b]	1.00 ± 0.05[a]	1.00 ± 0.03[a]	1.00 ± 0.00[a]	1.00 ± 0.02[a]	2.00 ± 0.03[b]	1.00 ± 0.00[a]
	MBC	0.50 ± 0.05[a]	4.00 ± 0.00[b]	4.00 ± 0.05[b]	4.00 ± 0.02[b]	4.00 ± 0.08[b]	2.00 ± 0.00[a]	4.00 ± 0.03[b]	8.00 ± 0.03[c]
Isoimperatorin	**MIC**	4.00 ± 0.09[a]	16.00 ± 0.11[c]	2.00 ± 0.08[a]	2.00 ± 0.05[a]	2.00 ± 0.07[a]	8.00 ± 0.09[b]	16.00 ± 0.07[c]	8.00 ± 0.07[b]
	MBC	8.00 ± 0.02[b]	n.i.	4.00 ± 0.00[a]	4.00 ± 0.07[a]	4.00 ± 0.08[a]	n.i.	n.i.	8.00 ± 0.04[b]
Acetyl cnidimol A	**MIC**	4.00 ± 0.03[a]	8.00 ± 0.09[b]	8.00 ± 0.03[b]	8.00 ± 0.02[b]	16.00 ± 0.02[c]	16.00 ± 0.04[c]	16.00 ± 0.06[c]	4.00 ± 0.03[a]
	MBC	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Streptomycin	**MIC**	0.09 ± 0.00[a]	0.17 ± 0.02[b]	0.17 ± 0.01[b]	0.04 ± 0.00[a]	0.17 ± 0.04[b]	0.17 ± 0.00[b]	0.26 ± 0.01[c]	0.17 ± 0.00[b]
	MBC	0.37 ± 0.02[a]	0.37 ± 0.00[a]	0.49 ± 0.03[a]	0.37 ± 0.02[a]	1.24 ± 0.00[c]	0.49 ± 0.03[a]	0.74 ± 0.07[b]	0.49 ± 0.03[a]

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

n.i. – not identified

830 **Table 8. Results of antifungal activity of *A. pancicii* Es and fluconazole in mg/mL (means ± SD)**

831

Fungi/ *A. pancicii* Es/Standard			*C. albicans*	*T. viride*	*P. ochrochloron*	*P. funiculosum*	*A. fumigatus*	*A. versicolor*	*A. ochraceus*	*A. niger*
ME	Aerial parts	MIC	3.00 ± 0.03[a]	6.00 ± 0.04[b]	6.00 ± 0.02[b]	6.00 ± 0.05[b]	6.00 ± 0.08[b]	6.00 ± 0.05[b]	8.00 ± 0.07[c]	8.00 ± 0.06[c]
		MFC	8.00 ± 0.02[a]	10.00 ± 0.02[a]	10.00 ± 0.10[a]	8.00 ± 0.08[a]	12.00 ± 0.07[a]	8.00 ± 0.08[a]	16.00 ± 0.04[b]	16.00 ± 0.02[b]
	Root	MIC	4.00 ± 0.07[a]	6.00 ± 0.03[b]	6.00 ± 0.03[b]	6.00 ± 0.02[b]	3.00 ± 0.05[a]	6.00 ± 0.04[b]	6.00 ± 0.05[b]	6.00 ± 0.07[b]
		MFC	14.00 ± 0.04[c]	10.00 ± 0.08[b]	10.00 ± 0.05[b]	10.00 ± 0.08[b]	4.00 ± 0.02[a]	10.00 ± 0.08[b]	12.00 ± 0.05[b]	12.00 ± 0.01[b]
EE	Aerial parts	MIC	8.00 ± 0.06[b]	6.00 ± 0.06[a]	8.00 ± 0.10[b]	6.00 ± 0.06[a]	8.00 ± 0.01[b]	8.00 ± 0.04[b]	6.00 ± 0.02[a]	8.00 ± 0.03[b]
		MFC	16.00 ± 0.04[b]	10.00 ± 0.02[a]	12.00 ± 0.11[a]	8.00 ± 0.07[a]	12.00 ± 0.02[a]	12.00 ± 0.05[a]	16.00 ± 0.07[b]	16.00 ± 0.05[b]
	Root	MIC	6.00 ± 0.02[a]	6.00 ± 0.11[a]	8.00 ± 0.08[b]	6.00 ± 0.04[a]	8.00 ± 0.00[b]	6.00 ± 0.03[a]	6.00 ± 0.03[a]	6.00 ± 0.01[a]
		MFC	8.00 ± 0.08[a]	10.00 ± 0.07[b]	10.00 ± 0.03[b]	8.00 ± 0.02[a]	10.00 ± 0.11[b]	8.00 ± 0.02[a]	12.00 ± 0.07[c]	12.00 ± 0.03[c]
AE	Aerial parts	MIC	14.00 ± 0.05[c]	12.00 ± 0.12[b]	14.00 ± 0.05[c]	10.00 ± 0.07[a]	10.00 ± 0.13[a]	10.00 ± 0.07[a]	10.00 ± 0.08[a]	14.00 ± 0.03[c]
		MFC	18.00 ± 0.08[b]	14.00 ± 0.05[a]	>18.00 ± 0.08[b]	>18.00 ± 0.05[b]	>18.00 ± 0.05[b]	>18.00 ± 0.10[b]	> 18.00 ± 0.09[b]	> 18.00 ± 0.07[b]
	Roots	MIC	14.00 ± 0.01[c]	12.00 ± 0.07[b]	10.00 ± 0.05[a]	10.00 ± 0.03[a]	10.00 ± 0.02[a]	10.00 ± 0.05[a]	14.00 ± 0.02[c]	10.00 ± 0.02[a]
		MFC	16.00 ± 0.03[a]	16.00 ± 0.08[a]	18.00 ± 0.09[b]	16.00 ± 0.10[a]	18.00 ± 0.08[b]	18.00 ± 0.03[b]	>18.00 ± 0.06[b]	> 18.00 ± 0.11[b]
Fluconazole		MIC	0.02 ± 0.01[a]	1.00 ± 0.01[c]	1.00 ± 0.07[c]	0.25 ± 0.00[a]	0.50 ± 0.02[b]	0.13 ± 0.02[a]	0.50 ± 0.00[b]	0.25 ± 0.03[a]
		MFC	0.03 ± 0.00[a]	1.50 ± 0.03[d]	1.50 ± 0.03[d]	0.50 ± 0.05[b]	1.00 ± 0.02[a]	0.50 ± 0.03[b]	1.00 ± 0.05[c]	1.00 ± 0.01[c]

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

833

834 **Table 9. Results of effects of *A. pancicii* Es, isolated compounds and standards (0.5MICs) on *P. aeruginosa* PAO1 twitching**
835 **and flagella motility and biofilm formation (%)**

836

Parameters/ *A. pancicii* Es/Standards		**Colony diameter (mm ± SE)**	**Flagella diameter (*μ*m)**	**Colony color**	**Colony edge**	**Biofilm formation***		
						0.5 MIC (% ± SE)	0.25 MIC (% ± SE)	0.125 MIC (% ± SE)
ME	Aerial parts	22.67 ± 2.52[b]	16 – 56	Light green	Tiny flagella	65.22 ± 1.87[c]	47.83 ± 1.06[b]	47.92 ± 1.37[c]
	Roots	20.67 ± 8.02[ab]	40 – 96	Green	Tiny flagella	52.43 ± 0.50[c]	40.38 ± 0.86[b]	34.54 ± 0.50[b]
EE	Aerial parts	23.67 ± 6.51[b]	56 – 128	Green	Regular flagella	53.58 ± 2.34[c]	54.73 ± 1.53[c]	43.77 ± 2.42[b]
	Roots	9.33 ± 1.53[a]	/	Light green	/	46.98 ± 2.37[b]	25.66 ± 0.58[ab]	17.36 ± 1.58[a]
AE	Aerial parts	23.00 ± 12.12[b]	40 – 160	Green	Regular flagella	62.26 ± 2.47[c]	74.15 ± 2.35[c]	31.70 ± 1.82[b]
	Roots	11.00 ± 1.00[a]	/	Light green	/	72.83 ± 0.85[c]	74.53 ± 2.21[c]	69.62 ± 1.06[cd]
tert–O–methyl oxypeucedanin hydrate		16.66 ± 7.64[a]	32	White	Tiny and reduced flagella	32.47 ± 1.07[b]	21.80 ± 0.44[a]	/
Oxypeucedanin		8.66 ± 4.04[a]	/	White	/	10.60 ± 0.53[a]	49.46 ± 0.93[bc]	/
Saxalin		23.33 ± 6.51[b]	112	White	Reduced flagella	52.17 ± 0.91[c]	32.40 ± 1.87[b]	57.88 ± 3.53[c]
Ostruthol		23.00 ± 5.57[b]	160	White	Regular flagella	30.64 ± 1.08[b]	11.06 ± 1.03[a]	40.35 ± 0.81[b]
Oxypeucedanin		15.33 ± 1.53[a]	80	White	Tiny flagella	41.44 ± 2.50[b]	37.30 ± 0.68[b]	2.72 ± 0.23[a]

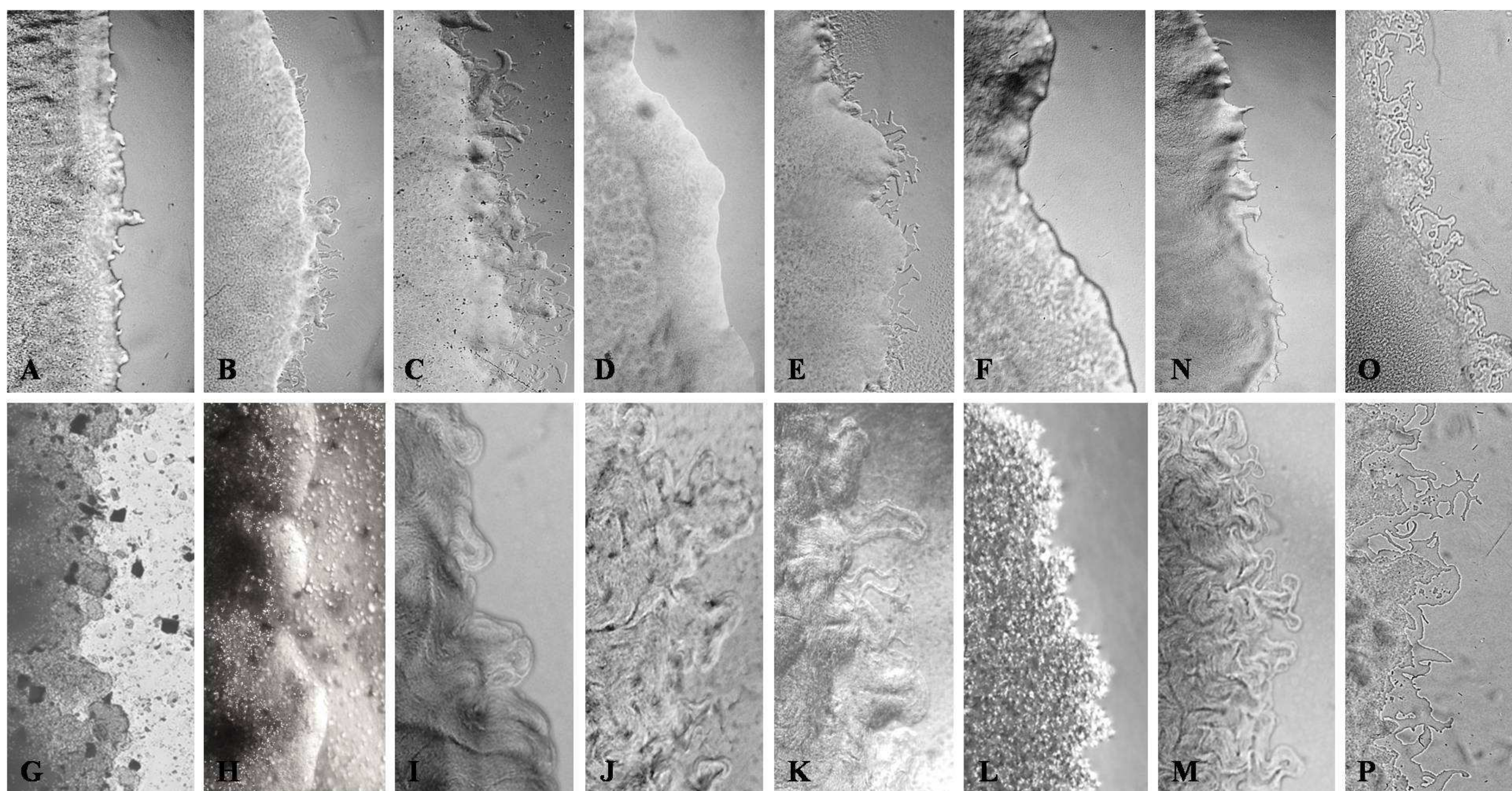
hydrate							
Isoimperatorin	46.66 ± 5.77^{c}	48	White	Tiny flagella	71.60 ± 1.93^{c}	57.68 ± 2.01^{c}	57.60 ± 3.17^{c}
Acetyl cnidimol A	28.00 ± 7.55^{b}	280	White	Regular flagella	35.05 ± 1.02^{b}	–	–
Streptomycin	11.00 ± 1.00^{a}	24 – 56	Green	Tiny flagella	69.16 ± 0.65^{c}	56.46 ± 0.46^{c}	92.16 ± 0.37^{d}
Ampicillin	13.33 ± 5.03^{a}	16 – 56	Green	Regular flagella	49.40 ± 0.46^{bc}	70.97 ± 0.36^{c}	88.36 ± 0.42^{d}
Control (10^9 CFU/mL)	12.00 ± 1.00^{a}	56 – 80	Light green	Regular flagella	/	/	/

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

Biofilm formation values were calculated as: ((mean A_{620} control well)/(mean A_{620} treated well)/mean A_{620} control well) x 100.

Values are expressed as means ± SD.

840

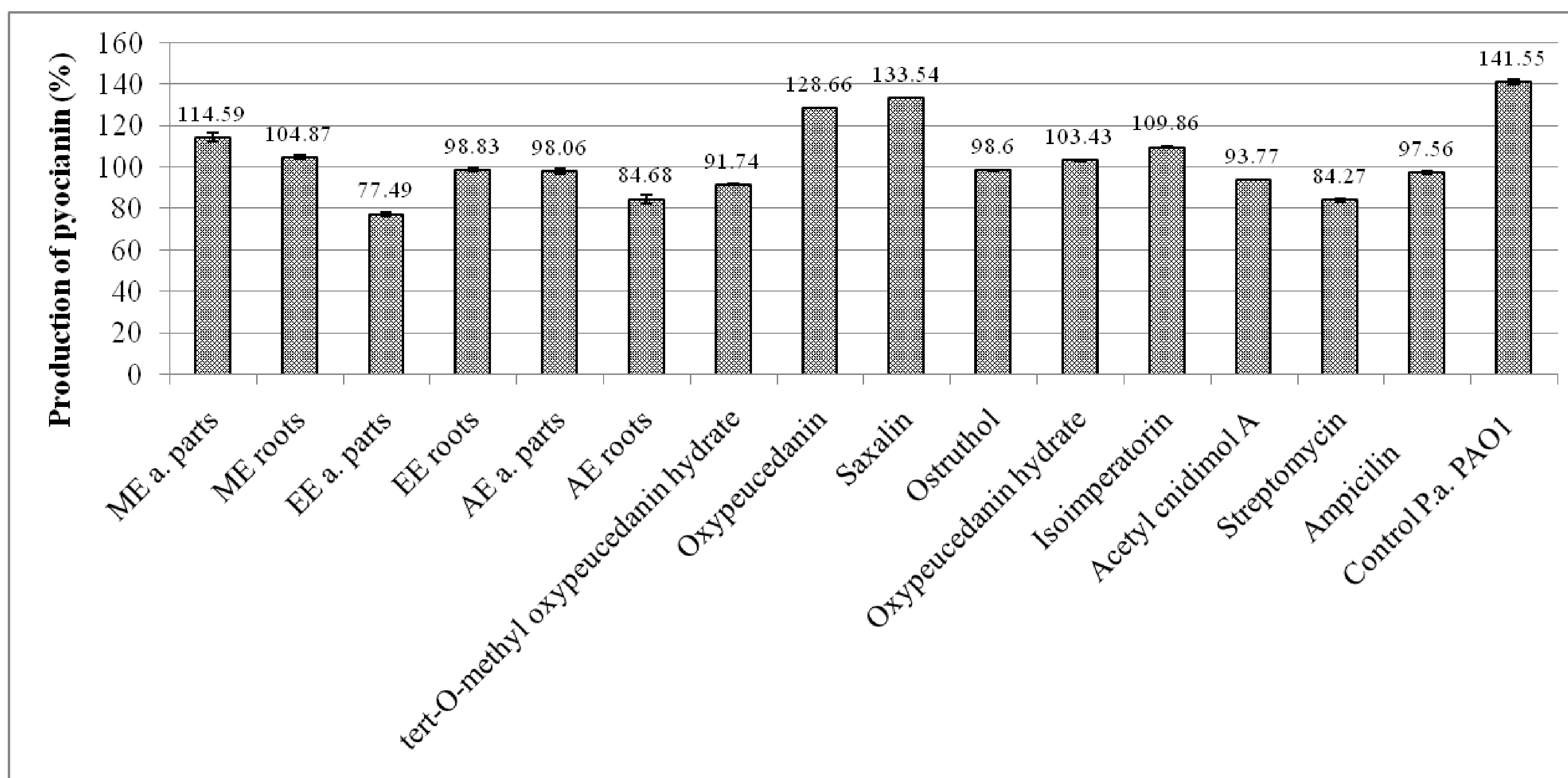


841 **Figure 2.** Light microscopy of colony edges of *P. aeruginosa* in twitching motility, grown in the presence of 0.5 MICs of *A. pancicii* Es,

842 isolated compounds and antibiotics. The bacterial colonies grown with the presence of Es (**A–F**); The bacterial colonies grown with the

843 presence of *A. pancicii* compounds (**G–M**); *P. aeruginosa* colony in the presence of streptomycin had reduced protrusion (**N**); *P.*

844 *aeruginosa* colony in presence of ampicillin with regularly formed protrusions (**O**); *P. aeruginosa* produced a flat, widely spread,
845 irregularly shaped colony in the absence of tested samples (**P**); Magnification: (**A–D**)×100.
846



847
848 **Figure 3.** Reduction of pyocyanin production of *P. aeruginosa* PAO1 by *A. pancicii* Es, isolated compounds and antibiotics tested at
849 0.5MICs (mg/mL).

84x27mm (150 x 150 DPI)