Supporting Information

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Flavonoids and Essential Oil of Bidens cernua of Polish Origin and In vitro Antimicrobial Activity of the Oil

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S1: *Gas chromatography-mass spectrometry (GC-MS) analysis:*

Qualitative and quantitative determination of the composition of the essential oil were performed by GC-MS analysis using polar and nonpolar columns on a Trace GC Ultra apparatus (Thermo Electron Corporation) equipped with a flame ionisation detector (FID) and MS DSQ II detectors and FID-MS splitter (SGE). An apolar capillary column Rtx-1ms (Restek Corporation) (60 m x 0.25 mm inner diameter with 0.25 µm film thickness) has been used with helium as a carrier gas at a regular pressure 200 kPa. The oven temperature was 50-300°C at a rate of 4°C/min. The SSL injector temperature was 280°C; FID temperature 300°C and split ratio 1:20. The polar capillary column HP-Innowax - 30 m x 0.25 mm, 0.25µm film thickness (Agilent J&W) was operated using SSL injector with temperature of 250°C and flame ionization detection (FID) temperature of 260°C, carrier gas (He) flow rate was 0.5

mL/min., split ratio 1:20. The oven temperature was programmed 50-245°C at 4°C/minute rate (30 minutes). Mass spectra were taken over the mass range 30-400 Da, ionization voltage was 70 eV and an ion source temperature of 200°C.

S2: *Identification of essential oil component:*

The chemical constituents of the essential oil were identified by using GC-FID-MS analysis on the basis of their mass spectra and retention indices, by comparison of literature data [1, 2] and mass spectral libraries (NIST 98.1, Wiley Registry of Mass Spectral Data, 8th Ed. and MassFinder 4). The percentage of each component within the essential oils was determined by peak area normalization measurements made without correction factors.

S3: *Antibacterial and antifungal activities of the essential oil:*

The biological activity of the essential oil of *B. cernua* has been tested on selected Gram-positive, Gram-negative bacteria and fungi. The minimum inhibitory concentration (MIC) of vancomycin and ciprofloxacin against tested bacterial strains and amphotericin B against fungal strains were estimated according to Clinical and Laboratory Standards Institute (NCCLS) standards. The methicillin-resistant *Staphylococcus* strains (MRSA) and bacterial strains producing extended-spectrum β-lactamase (ESBL) were also marked according to binding recommendation [3]. Broth microdilution assays [4, 5] were performed according to the NCCLS standards [3] with minor modifications to determine the minimum inhibitory concentration (MIC) of essential oil of *B. cernua* herb. The MIC values received for the essential oil are listed in Table 1.

Table 1. Minimum inhibitory concentration (MIC) of the essential oil of *B. cernua* herb against different microorganisms

Microorganisms	**Essential oil (MIC µg/mL)**	**Antibiotic MIC (µg/mL)**
Gram-positive bacteria		**Vancomycin**
Staphylococcus aureus ATCC 25923	6.2	0.75
Staphylococcus aureus (MSSA)* (n=4)	6.25±3.13	0.45±0.13
Staphylococcus aureus (MRSA)* (n=4)	9.38±3.125	0.87±0.38
Enterococcus faecalis ATCC 29212	100	3.1
*Enterococcus faecalis** (n=4)	46.88±28.13	1.13±0.63
*Streptococcus pyogenes**(n=3)	16.67±5.6	0.29±0.14
*Streptococcus pneumoniae**(n=4)	18.5±7.5	0.25±0.13
*Streptococcus agalactiae**(n=3)	14.58±6.94	0.25±0.17
Gram-negative bacteria		**Ciprofloxacin**
Klebsiella pneumoniae ATTC 700603 (ESBL+)	>100	0.75
*Klebsiella pneumoniae**(n=2)	>100	0.52±0.19
Klebsiella pneumoniae (ESBL+)* (n=2)	>100	0.67±0.11
Escherichia coli ATCC 25922	>100	0.16
Escherichia coli ATCC 35218 (β-lactamase +)	>100	0.75
Escherichia coli (ESBL+)*(n=3)	>100	0.73±0.18
*Proteus mirabilis**(n=2)	>100	0.42±0.12
Pseudomonas aeruginosa ATCC 27853	100	0.75
*Pseudomonas aeruginosa**(n=3)	54.17±30.56	0.35±0.1
*Neisseria gonorrhoeae**	1.56	0.008
*Moraxella catarrhalis**(n=4)	2.07±0.64	0.028±0.02
Fungi		**Amphotericin B**
Candida albicans ATCC 90028	12.5	0.5
*Candida albicans**(n=3)	16.67±5.56	0.29±0.14
Candida parapsilosis ATCC 22019	6.2	0.25
*Candida krusei**	50	0.5
*Candida glabrata**	25	0.25
*Geotrichum candidum**	3.1	0.12
*Cryptococcus neoformans**	6.2	0.5
*Rhodothorula rubra**	6.2	0.5

n - number of tested isolates
MIC - arithmetical means and average deflection of MIC value from "n" numbers of tested strains
* - microorganisms isolated from the clinical samples collected from selected patients of the University Clinical Hospital (USK) in Bialystok

S4: References

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