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Investigation of antimicrobial activity of *Pelargonium radula* (Cav.) L'Hérit

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Antimicrobial activities of two ethanolic extracts, made from fresh and dried leaves of *Pelargonium radula* (Cav.) L'Hérit, were tested against fourteen species of bacteria and fifteen species of fungi. The well-diffusion method indicated the strongest activity against *Pseudomonas aeruginosa*. The broth dilution method revealed that the most sensitive microorganisms were *Bacillus pumilus*, *Bacillus subtilis*, *Escherichia coli* and *Serratia marcescens*. Extract prepared from fresh leaves showed significantly higher antimicrobial activity than the extract prepared from dried leaves.

Keywords: *Pelargonium radula*, ethanolic extract, well-diffusion method, dilution method, antimicrobial activity

Pelargonium radula (Cav.) L'Hérit (*Geraniaceae*) is a decorative plant that originates from the Southeast of Africa. In literature, it is frequently found under different synonyms (*P. radens* H. E. Moore, *P. raduloides* Hoffmgg., *P. multifidum* Salisb., *P. revolutum* Jacq., *P. roseum* Wild., non Ait., Eckl. & Zeyh.). It is commercially cultivated for its essential oil. The main component of the essential oil is geraniol (up to 50%), while other important monoterpenes include citronellol, *i*-menthone, citronellyl formate, and geranyl formate, their oxides and esters (1).

Water and ethanolic extracts of the plant were found to have hypoglycaemic properties (2, 3). There are also indications that the plant extracts possess antimicrobial activity (4, 5). However, this activity has been investigated only on a small number of microorganisms. In the present study, we have further analyzed the antimicrobial activity of ethanolic extracts prepared from fresh as well as dried plants on 29 microorganisms.

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EXPERIMENTAL

Extracts preparation

Pelargonium radula was cultivated in the Botanical Garden of the Faculty of Pharmacy and Biochemistry in Zagreb. The voucher sample of the plant is deposited in the Herbarium of the Department of Botany, University of Zagreb, Croatia. Plant extracts were obtained by two procedures. Extract I (E1) was obtained from fresh leaves by percolation with 45% ethanol, and Extract II (E2) by percolation of dried leaves with 90% ethanol. Plant material was macerated for 12 h prior to percolation. For preparation of both extracts, 35 g of plant material was taken and 35 g of extract was collected.

Thin-layer chromatographic analysis

TLC analysis was carried out on Kieselgel 60 F_{254} (precoated 0.2 mm thick plastic plates, Merck, Germany) using the mobile phase: ethyl acetate/formic acid/acetic acid/water (100:11:11:27, *V/V*). Visualisation of flavonoids and phenolic acids was achieved by spraying the sheet with 1% methanolic solution of diphenylboric acid aminoethyl ester, followed by 5% ethanolic solution of polyethylene glycol 4000. The chromatogram was evaluated under UV light at 365 nm (6). Apigenin, luteolin, quercitrin, isoquercitrin, rutin and hyperoside and chlorogenic acid (Roth, Germany) were used for reference purposes.

Determination of total phenolics in the extracts

The amount of total phenolic compounds was determined spectrophotometrically according to Swain and Hills (7), and the amount of tannins was calculated according to the method of Schneider (8). Briefly, 2 mL of plant extract, previously diluted with methanol, was pipetted into a flask containing 10 mL of sodium acetate buffer and 8 mL of distilled water (Solution 1). To 10 mL of solution 1, 50 mg of casein was added, and the mixture was then vortexed for 45 minutes and filtrated (Solution 2). To 1 mL of each solution, 0.5 mL of Folin and Ciocalteu's phenol reagent was added, and the solutions were diluted to 10 mL with saturated sodium carbonate solution. Absorbance measurements were recorded at 725 nm and tannic acid was used in the construction of the standard curve. The amount of tannins was calculated as the difference in the amount of phenolic compounds before and after adsorption to casein. Estimation was carried out in triplicate.

Analysis of the flavonoid content in extracts

Flavonoid content was determined spectrophotometrically, according to the method of Christ and Müller (9). In short, 10 mL of acetone, 2 mL of 25% hydrochloric acid and 1 mL of 0.5% solution of hexamethylenetetramine was added to 10 mL of the extracts. After heating at 56 °C for 30 minutes, samples were cooled and diluted to 100 mL. 20 mL of each extract was diluted with water, and extracted three times with ethyl acetate. Organic layers were combined, washed twice with water, dried over anhydrous sodium

sulfate, and diluted to 50 mL. To 10 mL of each extract, 2 mL of 2% aluminum chloride solution in 5% methanolic solution of acetic acid was added. The samples were diluted to 25 mL with 5% methanolic solution of acetic acid. Absorbances (*A*) were read after 30 minutes at 425 nm. An extract solution prepared in the same manner but without addition of aluminum chloride solution served as a blank. Experiments were performed in triplicate.

Flavonoid content (x) expressed as quercetin percent was calculated from the equation

$$x = \frac{A \quad 0.772}{b}$$

where *b* is the mass of the extract, in grams.

Microbial strains

Six Gram-positive (*Bacillus cereus* ATCC 11778, *Bacillus pumilus* NCTC 8241, *B. subtilis* NCTC 8236, *Sarcina lutea* ATCC 9341, *Staphylococcus aureus* ATCC 6538P, *Enterococcus faecalis* ATCC 29212) and eight Gram-negative (*Escherichia coli* 923 MFBF, *Klebsiella oxytoca* MFBF, *Salmonella* sp. 1993 MFBF, *Shigella sonnei* MFBF, *Serratia marcescens* MFBF, *Citrobacter freundii* 051 MFBF, *Proteus rettgeri* MFBF, *Pseudomonas aeruginosa* ATCC 27895) bacterial strains were used in the study. Furthermore, acivity against eight yeast strains (*Candida albicans* MFBF, *C. apicola* MFBF, *C. guilliermondii* MFBF, *C. krusei* MFBF, *C. parapsilosis* MFBF, *C. kefyr* MFBF, *C. tropicalis* MFBF, *Hansenula anomala* MFBF), five moulds (*Aspergillus flavus* 3769 MFBF, *A. niger* 259 MFBF, *A. ochraceus* MFBF, *Penicillium* sp. MFBF) as well as three dermatophytes (*Microsporum gypseum* 539 MFBF, *Trichophyton mentagrophytes* 981 MFBF, *Epidermophytom floccosum* 951 MFBF) was tested. All tested species denoted with MFBF were from the collection of the Department of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia.

Diffusion method

Fresh cultures of bacterial strains were used for inoculum preparation. Before preparation, bacterial strains were cultured on Müller-Hinton agar (Merck, Germany) for 18 h at 37 °C with physiological saline. Density of inoculums used was 10^6 cells mL^{-1} as compared to MacFarland's standard solution of $BaSO_4$ (0.1 mL of 1% $BaCl_2$ + 9.9 mL of 1% H_2SO_4). Yeasts were cultivated on Sabouraud 2%-dextrose agar (Biolife, Italy) with the addition of chloramphenicol (50 mg L^{-1}) for 5 days at 25 °C for suppression of bacterial growth. One mL of inoculum was mixed with approximately 16 mL of melted agar, previously cooled down to 45 °C. Müller-Hinton or Sabouraud 2%-dextrose agar were used for bacterial or yeast strains, respectively. Inoculated agars were then cooled to room temperature. The wells (6 mm in diameter) were cut from the agar with stainless steel cylinders and 0.2 mL of extract solution (E1 or E2) or 70% ethanol was delivered into them. To ensure enough time for diffusion of samples into agar, plates were incubated at 4 °C for 1 h. They were then incubated at either 37 °C for bacteria or 25 °C for yeasts for appropriate time periods under aerobic conditions. The diameter of the inhibition zone around each hole was measured and recorded.

Dilution method

For determination of minimum inhibitory concentration (*MIC*) and minimum microbicidal concentration (*MMC*), the broth twofold macrodilution method in Müller-Hinton or Sabouraud broth (Merck, Germany) was applied. In short, test strains were grown in a nutrition medium containing progressively lower dilutions of the test extract and incubated at 37 °C for bacteria or 25 °C for yeasts. The last two tubes were free of test extract and served as growth control in broth and 70% ethanol. After incubation, approximately the 10 μL of content of each test-tube was transfered with a loop onto Müller Hinton or Sabouraud agar. Agar plates were incubated for an appropriate time under aerobic conditions at 37 °C or 25 °C for bacteria or yeasts, respectively. *MIC* was defined as the lowest concentration of extract that allows no more than 20% bacterial growth, and *MMC* as the lowest extract concentration from which the microorganisms did not recover and grow when transferred to fresh medium.

RESULTS AND DISCUSSION

The presence of flavonoids quercitrin, isoquercitrin, rutin and hyperoside as well as chlorogenic acid in both extracts was confirmed by the TLC method. The results of spectrometric determination showed that ethanolic extract differed in the amount of phenolic compounds. Extract prepared from fresh leaves with 45% ethanol (E1) contained 0.453 ± 0.001% (mean ± SD, n = 3) and the extract prepared from dried leaves (E2) 0.228 ± 0.011% (mean ± SD, n = 3) of phenols. The amount of tannins in E1 was found to be 0.206 ± 0.005% (mean ± SD, n = 3), while E2 contained 0.080 ± 0.015% (mean ± SD, n = 3) of these compounds. The content of flavonoids was also different. E1 contained 0.018 ± 0.001% (mean ± SD, n = 3), while E2 contained 0.028 ± 0.001% (mean ± SD, n = 3) of flavonoids.

Ethanolic extracts of *Pelargonium radula* were screened for antimicrobial activity against 14 bacterial, 8 yeast, 5 mould and 3 dermatophyte strains by the agar diffusion method (Table I). 70% ethanol, which was used as the control, showed no zones of inhibition. Extract E1 was active against all the bacterial strains tested. Among the microorganisms tested, *Pseudomonas aeruginosa* was the most susceptible to the extract, inhibition zone being 22 mm. E1 was active against all the bacterial strains included in testing. Inhibition zones ranged from 11–15 mm for a vast majority of bacteria. The activity against yeasts and moulds was of somewhat reduced spectrum. Only *Candida guilliermondii*, *C. parapsilosis* and *C. kefyr* were moderately sensitive to E1. On the other hand, E1 exerted activity against two of the three dermatophytes included in investigation. In contrast to E1, which exhibited activity of a relatively broad spectrum, extract E2 was inactive against the majority of the microorganisms tested. The bacterium most susceptible to extract E1 was *Pseudomonas aeruginosa*. The inhibition zone around the well was 15 mm.

The results of the broth twofold macrodilution method for E1 are presented in Table II. The procedure was performed only on microorganisms that were found to be sensitive to the extracts in the diffusion method. All the tested bacteria were inhibited by extract concentrations ranging from 3.13 to 60%. Among the Gram-positive bacteria, the strongest effect of the extract (*MIC* 3.13%) was observed against *Bacillus pumilus* and *Bacillus subtilis*. As regards Gram-negative strains, E1 demonstrated the lowest *MIC* against *Escherichia coli*

Table I. Results of the well-diffusion method for E1 and E2

Microorganism	Inhibition zone (mm)	
	E1	E2
Bacillus cereus ATCC 11778	14	12
Bacillus pumilus NCTC 8241	11	–
Bacillus subtilis NCTC 8236	14	11
Sarcina lutea ATCC 9341	8	8
Staphylococcus aureus ATCC 6538P	12	12
Enterococcus faecalis ATCC 29212	12	–
Escherichia coli 923 MFBF	7	–
Klebsiella oxytoca MFBF	12	–
Salmonella sp. 1993 MFBF	14	–
Shigella sonnei MFBF	15	–
Serratia marcescens MFBF	12	–
Citrobacter freundii 051 MFBF	15	–
Proteus rettgeri MFBF	14	–
Pseudomonas aeruginosa ATCC 27895	22	15
Candida albicans MFBF	–	–
Candida apicola MFBF	–	–
Candida guilliermondii MFBF	10	–
Candida krusei MFBF	–	–
Candida parapsilosis MFBF	8	–
Candida kefyr MFBF	–	–
Candida tropicalis MFBF	7	–
Hansenula anomala MFBF	–	–
Aspergillus flavus 3769 MFBF	–	–
Aspergillus niger 259 MFBF	–	–
Aspergillus ochraceus MFBF	–	–
Penicillium sp. MFBF	–	11
Microsporum gypseum 539 MFBF	7	–
Trichophyton mentagrophytes 981 MFBF	6	–
Epidermophytom floccosum 951 MFBF	–	–

ATCC – American Type Culture Collection, Rockville, USA; NCTC – National Collection of Type Cultures, London, Great Britain; MFBF – collection of microorganisms of the Department of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia.

and *Serratia marcescens*, while the growth of *Klebsiella oxytoca*, *Salmonella* sp., *Shigella sonnei* and *Citrobacter freundii*, was inhibited by the 6.25% concentration. The only yeast tested by the dilution method, *Candida guilliermondii* was inhibited by E1 concentration of 6.25%, while *Microsporum gypseum* and *Trichophyton mentagrophytes* were inhibited by the concentration of 12.5%. The lowest *MMC* (6.25%) among all the microorganisms included in the dilution method was observed for *Bacillus pumilus*, *Bacillus subtilis*, *Escherichia coli* and *Serratia marcescens*. Table II shows the results of the dilution method for E2. In addition to its limited spectrum of activity, extract E2 demonstrated relatively high *MIC*s for all the microorganisms included in the dilution method. The most sensitive one was *Staphylococcus aureus* (*MIC* 20%). For the other microorganisms tested *MIC*s ranged from 25 to 50%.

Table II. Results of the dilution method for E1

Microorganism	Concentration (%)	
	MIC[a]	*MMC*[b]
Bacillus cereus ATCC 11778	25	50
Bacillus pumilus NCTC 8241	3.13	6.25
Bacillus subtilis NCTC 8236	3.13	6.25
Sarcina lutea ATCC 9341	50	60
Staphylococcus aureus ATCC 6538P	20	25
Enterococcus faecalis ATCC 29212	12.5	25
Escherichia coli 923 MFBF	3.13	6.25
Klebsiella oxytoca MFBF	6.25	12.5
Salmonella sp. 1993 MFBF	6.25	12.5
Shigella sonnei MFBF	6.25	12.5
Serratia marcescens MFBF	3.13	6.25
Citrobacter freundii 051 MFBF	6.25	12.5
Proteus rettgeri MFBF	10	12.5
Pseudomonas aeruginosa ATCC 27895	25	50
Candida guilliermondii MFBF	6.25	12.5
Microsporum gypseum 539 MFBF	12.5	25
Trichophyton mentagrophytes 981 MFBF	12.5	25

[a] Minimum inhibitory concentration.
[b] Minimum microbicidal (bactericidal or fungicidal) concentration.

Table III. Results of the dilution method for E2

Microorganism	Concentration (%)	
	MIC[a]	*MMC*[b]
Bacillus cereus ATCC 11778	25	50
Bacillus subtilis NCTC 8236	30	50
Sarcina lutea ATCC 9341	50	60
Staphylococcus aureus ATCC 6538P	20	25
Pseudomonas aeruginosa ATCC 27895	25	50
Penicillium sp. MFBF	50	60

[a] Minimum inhibitory concentration
[b] Minimum microbicidal (bactericidal or fungicidal) concentration

CONCLUSIONS

While the efficacy of the dried-leaves extract was found to be moderate, the fresh--leaves extract demonstrated significant antimicrobial activity. Comparison of the flavonoid content of the extracts shows that E2 is richer in these compounds than E1. Therefore, it

might be concluded that flavonoids are not the main active compounds of the extracts, but rather other phenolic compounds and/or essential oil. Results reported here contribute to the knowledge of the antimicrobial efficacy of *Pelargonium radula*.

REFERENCES

1. Z. Kalođera, N. Blažević and M. Volenec, Composition of the essential oil of *Pelargonium radula* (Cav.) L'Herit: A study of its vegetative cycle, *Acta Pharm.* **51** (2001) 153–157.
2. M. Sljepčević, Z. Kalođera and M. Hadžija, Hypoglycaemic effect of *Pelargonium radula* (Cav.) L'Herit, *Acta Pharm.* **47** (1997) 101–108.
3. Z. Kalođera, M. Hadžija, M. Slijepčević and P. Theiss, Stimulation of DNA synthesis in isolated Langerhans islets treated by *Pelargonium radula* (Cav.) L'Herit. Extracts with hypoglycaemic efficacy, *Acta Pharm.* **49** (1999) 181–188.
4. H. Wollmann, G. Habicht, I. Lau and I. Schultz, Various properties of the volatile oils of *Pelargonium roseum* Willd. from indigenous cultivation, *Pharmazie* **28** (1973) 56–58.
5. M. Lis-Balchin, G. Buchbauer, K. Ribisch and M.-T. Wenger, Comparative antibacterial effects of novel *Pelargonium* essential oils and solvent extracts, *Lett. Appl. Microbiol.* **27** (1998) 135–141.
6. H. Wagner, S. Bladt and E. M. Zgainski, *Drogenanalyse*, Springer Verlag, Berlin 1983, pp. 163– 165.
7. T. Swain and W. E. Hills, The phenolic constituents of *Prunus domestica* I. – The quantitative analysis of phenolic constituents, *J. Sci. Food Agric.* **10** (1959) 63–68.
8. G. Schneider, Zur Bestimmung der Gerbstoffe mit Casein, *Arch. Pharm.* **309** (1976) 38–44.
9. B. Christ and K. H. Müller, Zur serienmäßigen Bestimmung des Gehaltes an Flavonol-Derivaten in Drogen, *Arch. Pharm.* **293** (1960) 1033–1042.

S A Ž E T A K

Ispitivanje antimikrobne aktivnosti *Pelargonium radula* (Cav.) L'Hérit

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Od svježih, odnosno suhih listova *Pelargonium radula* (Cav.) L'Hérit, pripravljena su dva etanolna ekstrakta. Antimikrobna aktivnost ekstrakata je ispitana na četrnaest bakterijskih vrsta i petnaest vrsta gljivica. Metodom difuzije najjače djelovanje pokazivao je ekstrakt na vrstu *Pseudomonas aeruginosa*. Najosjetljiviji mikroorganizmi u metodi dilucije bili su *Bacillus pumilus*, *Bacillus subtilis*, *Escherichia coli* i *Serratia marcescens*. Ekstrakt pripravljen od svježih listova pokazao je znatno veću antimikrobnu aktivnost od ekstrakta priređenog od suhih listova.

Ključne riječi: *Pelargonium radula*, etanolni ekstrakt, metoda difuzije, metoda dilucije, antimikrobna aktivnost

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