Acta Pharm. 55 (2005) 431–435 Short communication

Antimicrobial activity of flavonoids from *Pelargonium radula* (Cav.) L'Hérit

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Received March 15, 2005
Accepted October 5, 2005

Flavonoids from *Pelargonium radula* (Cav.) L'Hérit were purified by column chromatography. Two fractions were obtained: F1 (main flavonoid isoquercitrin) and F2 (main flavonoid rutin). *In vitro* antimicrobial activity of F1 and F2 were tested against eleven species of bacteria and eleven species of fungi. Both fractions demonstrated strong inhibitory activity against *Staphylococcus aureus*, *Proteus rettgeri*, *Candida tropicalis* and *Microsporum gypseum*. *Staphylococcus* sp. (coagulase-negative) and *Candida lusitaniae* were strongly inhibited only by fraction F1 and *Fusarium graminearum* only by fraction F2.

Keywords: *Pelargonium radula*, antimicrobial activity, flavonoids, rutin, isoquercitrin, well-diffusion method, dilution method

Flavonoids are a group of natural compounds known to have various pharmacological properties such as antioxydative, antiinflammatory and diuretic (1). One of the well--known actions of flavonoids is antimicrobial activity. *Pelargonium radula* (Cav.) L'Hérit (*Geraniaceae*) is a plant rich in essential oil, which also contains flavonoids. While the antimicrobial activity of the whole extract of *Pelargonium radula* (2, 3) and isolated essential oil (4) has been investigated in more detail, the flavonoid contribution to overall antimicrobial activity has remained unclear. In the present study we have investigated the antimicrobial activity of *Pelargonium radula* flavonoids against 22 microorganisms.

EXPERIMENTAL

Preparation of flavonoid fractions

Extract of dried *Pelargonium radula* leaves, grown in the Botanical Garden of the Faculty of Pharmacy and Biochemistry in Zagreb, was prepared by percolation. The voucher sample of the plant is deposited in the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia.

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Plant material was macerated for 12 h with 96% ethanol and percolated with the same solvent. The percolate was collected, evaporated and the oily residue was subjected to column chromatography on silica gel (Kemika, Croatia) with ethyl acetate/formic acid/acetic acid/water (100:11:11:27, *V*/*V*) as eluent. Two main fractions were collected (F1 and F2), evaporated and re-dissolved in ethanol (70%). Flavonoid content of each fraction was determined spectrophotometrically according to the method of Christ and Müller (5) and adjusted to 0.2% of flavonoids by addition of the same solvent.

Thin-layer chromatographic analysis

Merck silica gel plates Kieselgel 60 F_{254} (Merck, Germany) and the mobile phase ethyl acetate/formic acid/acetic acid/water (100:11:11:27, *V*/*V*) were used for TLC. The chromatogram was evaluated under UV light at 365 nm (6) after spraying the plate with NST-PEG reagent (1% methanolic solution of diphenylboric acid aminoethyl ester, followed by 5% ethanolic solution of polyethylene glycol 4000). Apigenin, luteolin, quercitrin, isoquercitrin, rutin, hyperoside and chlorogenic acid (Roth, Germany) were used as reference substances.

Antimicrobial assays

Microorganisms included in the study were *Bacillus cereus* ATCC 11778, *B. pumilus* NCTC 8241, *B. subtilis* NCTC 8236, *Sarcina lutea* ATCC 9341, *Staphylococcus* sp. (coagulase-negative) MFBF, *S. aureus* ATCC 6538P, *Escherichia coli* 923 MFBF, *Klebsiella oxytoca* MFBF, *Proteus rettgeri* MFBF, *Salmonella* sp. 1993 MFBF, *Shigella sonnei* MFBF, *Candida albicans* MFBF, *C. glabrata* MFBF, *C. krusei* MFBF, *C. lusitaniae* MFBF, *C. parapsilosis* MFBF, *C. tropicalis* MFBF, *Aspergillus ochraceus* MFBF, *Fusarium graminearum* MFBF, *Epidermophytom floccosum* 951 MFBF, *Microsporum gypseum* 539 MFBF and *Trichophyton mentagrophytes* 981 MFBF. 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. Antimicrobial activity was investigated by the well-diffusion method and the broth two-fold macrodilution method. Results of the diffusion method were expressed as the diameter of the inhibition zone around the hole filled with investigated solution. Dilution method results were recorded as the minimum inhibitory concentration (*MIC*) and minimum microbicidal concentration (*MMC*). Details of both methods are described elsewhere (2).

RESULTS AND DISCUSSION

Flavonoids of *Pelargonium radula* were separated in two main fractions, which were evaporated, redissolved in 70% ethanol and adjusted to 0.2% of flavonoids by addition of the same solvent. TLC chromatography was employed to determine the composition of flavonoids in each fraction. Isoquercitrin was the main flavonoid in F1 and rutin in F2. F1 also contained hyperoside, while chlorogenic acid was present in both fractions.

Antimicrobial activity of both fractions was investigated on 11 bacterial, 6 yeast, 2 mould and 3 dermatophyte strains by the well-diffusion method (Table I). The control

Table I. Diameters of inhibition zones in the well-diffusion method

Microorganism	Inhibition zone (mm)	
	F1	F2
Bacillus cereus ATCC 11778	9	9
Bacillus pumilus NCTC 8241	9[a]	12[a]
Bacillus subtilis NCTC 8236	12	13
Sarcina lutea ATCC 9341	11	12[a]
Staphylococcus sp. (coagulase-negative) MFBF	9[a]	9[a]
Staphylococcus aureus ATCC 6538P	11	13
Escherichia coli 923 MFBF	10[a]	11[a]
Klebsiella oxytoca MFBF	11	–
Proteus rettgeri MFBF	9[a]	10[a]
Salmonella sp. 1993 MFBF	13	12
Shigella sonnei MFBF	–	–
Candida albicans MFBF	–	–
Candida glabrata MFBF	–	–
Candida krusei MFBF	–	–
Candida lusitaniae MFBF	–	8
Candida parapsilosis MFBF	–	–
Candida tropicalis MFBF	10[a]	10[a]
Aspergillus ochraceus MFBF	–	–
Fusarium graminearum 373 MFBF	13	9
Epidermophytom floccosum 951 MFBF	14	12
Microsporum gypseum 539 MFBF	14	11
Trichophyton mentagrophytes 981 MFBF	12[a]	–

[a] microbiostatic action
ATCC – American Type Culture Collection, Rockville, USA.
NCTC – National Collection of Type Cultures, London, Great Britain.
MFBF – number of strains from the Collection of microorganisms of the Department of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia.

(70% ethanol) showed no inhibition zones. Both flavonoid fractions inhibited the growth of most of the bacterial strains tested. The only exceptions were *Shigella sonnei* (F1 and F2) and *Klebsiella oxytoca* (F2). In general, F2 exerted somewhat stronger action against most of the bacterial strains than F1. On the other hand, the majority of yeasts were resistant to both flavonoid fractions. *Candida tropicalis* was the only yeast inhibited by both extracts, while F2 exerted microbicidal action against *Candida lusitaniae*. Among moulds, *Fusarium graminearum* was sensitive to both fractions. All the dermatophyte strains were sensitive to both fractions, the only exception being *Trichophyton mentagrophytes*, which was resistant to F2.

The size of all inhibition zones was between 8 and 14 mm. Average size of inhibition zones was around 11 mm for both fractions. Microorganisms most susceptible to F1 were *Epidermophytom floccosum* and *Trichophyton mentagrophytes* (inhibition zones 14 mm), while F2 demonstrated the strongest action against *Staphylococcus aureus* and *Bacillus subtilis* (inhibition zone 13 mm).

Only the microorganisms that were sensitive to F1 or F2 were included in investigations by the dilution method (Table II). For the vast majority of microorganisms, both

Table II. Minimum inhibitory and microbicidal concentrations of F1 and F2

Microorganism	Concentration (%)			
	F1		F2	
	MIC[a]	*MMC*[b]	*MIC*[a]	*MMC*[b]
Bacillus cereus ATCC 11778	> 50	> 50	–	–
Bacillus pumilus NCTC 8241	> 50	> 50	12	15
Bacillus subtilis NCTC 8236	12	14	25	30
Sarcina lutea ATCC 9341	8	10	21	23
Staphylococcus sp. (coagulase-negative) MFBF	2	8	18	20
Staphylococcus aureus ATCC 6538P	6	7	6	6
Escherichia coli 923 MFBF	12	15	13	14
Klebsiella oxytoca MFBF	12	18	16	18
Proteus rettgeri MFBF	5	6	5	6
Salmonella sp. 1993 MFBF	10	13	14	16
Candida lusitaniae MFBF	–	–	7	9
Candida tropicalis MFBF	6	6	5	6
Fusarium graminearum 373 MFBF	9	13	5	7
Epidermophytom floccosum 951 MFBF	9	12	9	12
Microsporum gypseum 539 MFBF	6	12	6	7
Trichophyton mentagrophytes 981 MFBF	15	11	–	–

[a] minimum inhibitory concentation
[b] minimum microbicidal (bactericidal or fungicidal) concentration
For other symbols see Table I.

fractions were microbicidal in concentrations of up to 20%. Average minimum inhibitory concentrations were 9% (F1) and 12% (F2), while average minimum microbicidal concentrations were 11% (F1) and 14% (F2). Microorganisms that were strongly inhibited by both fractions (active concentration less than 7%) were *Staphylococcus aureus*, *Proteus rettgeri*, *Candida tropicalis* and *Microsporum gypseum*. *Staphylococcus* sp. (coagulase-negative) and *Candida lusitaniae* were strongly inhibited only by F1, and *Fusarium graminearum* only by F2.

CONCLUSIONS

Although flavonoids are not the main active principle of *Pelargonium radula*, the results reported here demonstrate that the main flavonoids, isoquercitrin and rutin, contribute significantly to the intensity and range of the overall antimicrobial activity of the plant. This may be of special interest in the cases of extracts prepared from dry plant material.

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S A Ž E T A K

Antimikrobna aktivnost flavonoida *Pelargonium radula* (Cav.) L'Hérit

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Flavonoidi biljne vrste *Pelargonium radula* (Cav.) L'Hérit pročišćeni su kromatografijom na stupcu. Dobivene su dvije glavne flavonoidne frakcije: F1 (vodeći flavonoid izokvercitrin) i F2 (vodeći flavonoid rutin). Dilucijskom i difuzijskom metodom određena je antimikrobna aktivnost F1 i F2 na jedanaest bakterijskih sojeva i jedanaest sojeva gljivica. Obje frakcije pokazale su snažno inhibicijsko djelovanje na sojeve *Staphylococcus aureus*, *Proteus rettgeri*, *Candida tropicalis* i *Microsporum gypseum*. Na sojeve *Staphylococcus* sp. (koagulaza-negativan) i *Candida lusitaniae* snažno je djelovala samo frakcija F1, a na *Fusarium graminearum* samo frakcija F2.

Ključne riječi: *Pelargonium radula*, antimikrobna aktivnost, flavonoidi, rutin, izokvercitrin, metoda difuzije, metoda dilucije

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