Antimicrobial Activity of *Gentiana lutea* L. Extracts

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Methanolic extracts of flowers and leaves of *Gentiana lutea* L., together with the isolated compounds mangiferin, isogentisin and gentiopicrin, were used to investigate the antimicrobial activity of the plant. A variety of Gram-positive and Gram-negative bacteria as well as the yeast *Candida albicans* has been included in this study. Both extracts and isolated compounds showed antimicrobial activity with MIC values ranging from 0.12–0.31 mg/ml. Our study indicated that the synergistic activity of the pure compounds may be responsible for the good antimicrobial effect of the extracts. Quantification of the secondary metabolites was performed using HPLC.

Key words: Gentiana lutea, Antimicrobial Activity, Gentiopicrin

Introduction

The roots of *Gentiana lutea* L. (Gentianaceae), a yellow flowering plant commonly found in the mountain regions of central and south Europe, are very popular as a stomachic as well as a component in preparations showing beneficial effects in gall and liver diseases (Wichtl, 1994). The active principles are the bitter tasting secoiridoid glycosides gentiopicrin and amarogentin. Some investigations pointed out an interesting chemical composition of the aerial parts of *G. lutea*. The presence of the xanthone isogentisin and two flavone heterosides was reported in leaves of *G. lutea* (Hostettmann *et al*., 1973). The secoiridoids gentiopicrin and swertiamarin, the xanthones mangiferin, isogentisin and isogentisin-3-*O*-primeveroside, and the flavones isoorientin and isovitexin have been isolated from the aerial parts of *G. lutea* (Menković *et al*., 2000).

The development of resistance by pathogens to many of the commonly used antibiotics provides a stimulus for further attempts to search for new antimicrobial agents to combat infections and overcome problems of resistance and side effects of the currently available antimicrobial agents. Antibacterial effects of *G. lutea* roots have been described recently, and it was shown that the dry extract was effective against *Streptococcus pyogenes* (Weckesser *et al*., 2007). Other studies indicated that *G. lutea* exhibits antimicrobial effects that correspond to the effect of ampicillin and it could be used in the treatment of bacterial infections (Stierna *et al*., 2005). As for the antimicrobial activity of leaves and flowers extracts is concerned, only an antitubercular effect against *Mycobacterium bovis* was reported (Menković *et al*., 1999). The aim of the present study was to investigate the antimicrobial activity of *G. lutea* leaves and flowers extracts and the isolated compounds gentiopicrin, isogentisin and mangiferin against various bacteria and the yeast *Candida albicans*.

Material and Methods

Plant material

Leaves and flowers of *Gentiana lutea* were collected at mountain Suvobor (at a height of ca. 830 m), Serbia, in July 2006. A voucher specimen (17506) has been deposited in the herbarium of the Botanic Garden "Jevremovac", Faculty of Biology, University of Belgrade, Serbia.

Sample preparation

Air-dried leaves and flowers were extracted separately with methanol (1000 ml) in a Soxhlet apparatus for 24 h and the solvent was evaporated. Dry extracts of leaves (17.5 g) and flowers (22.3 g) were used for the experiments.

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HPLC conditions

Analyses were carried out on a HP series 1090 instrument with a DAD detector, on a reverse phase Zorbax SB-C18 analytical column [150 × 4.6 mm i.d., particle size 5 μm (Agilent)]. Mobile phase A was H_2O containing 1% 0.1 N H_3PO_4, mobile phase B was MeCN. Gradient elution was according to the following scheme: 98–90% A, 0–5 min; 90% A, 5–10 min; 90–85% A, 10–13 min; 85% A, 13–15 min; 85–70% A, 15–20 min; 70–40% A, 20–24 min; 40–0% A, 24–28 min; flow at 1 ml/min; detection at 260 and 320 nm. The xanthones mangiferin and isogentisin, and the secoiridoid gentiopicrin were isolated according to the previously published procedure (Menković *et al*., 2000). Quantification was performed using HPLC and the amounts of the compounds were calculated using calibration curves. All experiments were repeated at least three times. The results are presented as mg/g of dry weight (dw).

Studied activity

The antimicrobial activity was tested against six Gram-negative (*Escherichia coli, Salmonella typhimurium, S. enteritidis, Pseudomonas aeruginosa*, *P. tolaasii*, *Enterobacter cloacae*) and nine Gram-positive bacteria (*Staphylococcus aureus*, *S. epidermidis, Streptococcus faecalis, Bacillus subtilis*, *Micrococcus luteus*, *M. flavus, Proteus mirabilis*, *Sarcina lutea*, *Listeria monocytogenes*), as well as one human pathogen yeast (*Candida albicans*). The MIC (minimum inhibitory concentration) values were determined using the broth microdilution method in 96-hole plates according to NCCLS (2000). Serial dilutions of the stock solutions of test extracts in broth medium (Muller-Hinton broth or Sabouraud broth) were prepared in a microtiter plate. The microbial suspensions were added in the microwells at the concentration of $5 \cdot 10^5$ organisms/ml. The MIC values were determined as the lowest concentrations preventing visible growth. Streptomycin and nystatin were used as a positive control. Each assay was repeated independently two times.

Results and Discussion

The HPLC profiles of *G. lutea* leaves and flowers methanolic extracts are shown in Fig. 1. The amounts of mangiferin and gentiopicrin were nearly similar in leaves and flowers, but the amount of isogentisin was about ten times higher in flowers (Table I).

The results of the antimicrobial activity determination of *G. lutea* extracts and isolated compounds are presented in Table II. Leaves and flowers extracts inhibited the growth of 15 of 16 pathogenic microorganisms tested, only the Gram-positive bacterium *Listeria monocytogenes* was resistant and has grown at the highest applied concentrations of both extracts. The MIC values of the leaves extract were between 0.12 and 0.31 mg/ml, and the most sensitive to this extract were *Pseudomonas aeruginosa, Bacillus subtilis*, *Proteus mirabilis, Staphylococcus epidermidis* and *Candida albicans*. The flowers extract exerted slightly lower antimicrobial activity and the most susceptible microorganism was *Salmonella enteritidis* (MIC 0.15 mg/ml).

Among the individual extract components, the compound with the widest spectrum of activity was found to be gentiopicrin. It was most active against *Escherichia coli* (0.12 mg/ml) and showed moderate activity against *Salmonella typhimurium* and *Staphylococcus aureus* (0.15 mg/ml). The obtained results, along with published data, characterize gentiopicrin as a natural compound with a broad antimicrobial effect (Kumarasamy *et al*., 2003; Nadinic *et al*., 2002). The antimicrobial activity of the xanthone isogentisin against *Mycobacterium bovis* has been reported previously (Menković *et al.*, 1999). In the present study, isogentisin showed moderate antimicrobial activities with MIC values between 0.15 and 0.31 mg/ml. Among the Gram-negative bacteria the most susceptible were *E. coli* and *Pseudomonas aeruginosa*. The species most sensitive to isogentisin among the Gram-positive bacteria was *Micrococcus luteus*. Compared to the other examined compounds, mangiferin showed lower antibacterial activity with MIC values between 0.20 and 0.31 mg/ml. The antimicrobial activity of mangif-

Table I. The amount of secondary metabolites in *G. lutea* leaves and flowers extracts.

Sample	Gentiopicrin[a] [mg/g dw]	Mangiferin[a] [mg/g dw]	Isogentisin[a] [mg/g dw]
Leaves	38.85 ± 0.7	9.57 ± 0.4	12.86 ± 0.7
Flowers	48.38 ± 1.4	8.98 ± 0.4	123.23 ± 3.1

[a] Mean ± s.d. (n = 3).

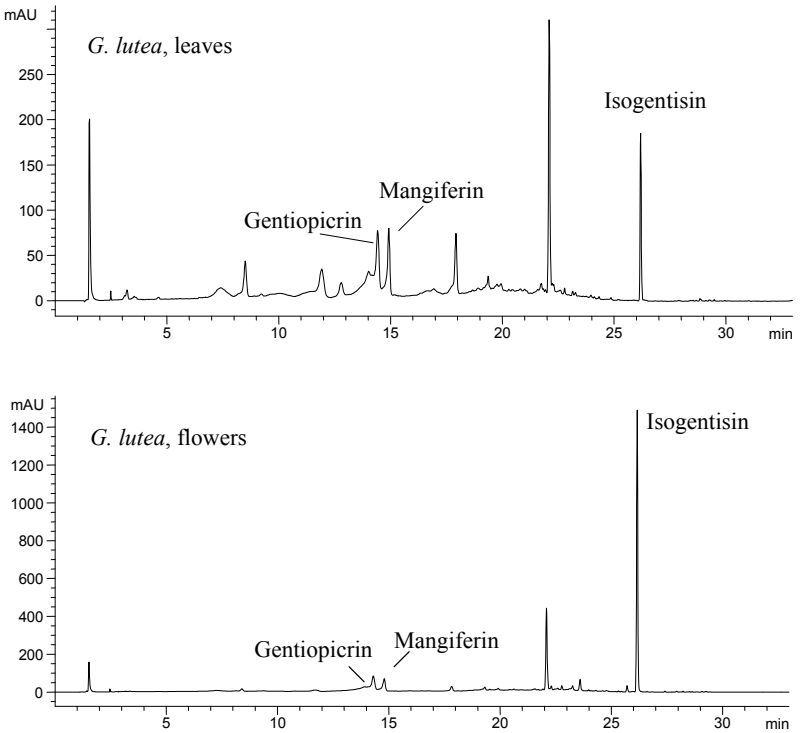

Fig. 1. HPLC chromatograms of methanolic extracts of *G. lutea* leaves and flowers.

Table II. Antimicrobial activity (MIC values in mg/ml) of *G. lutea* leaves and flowers extracts and isolated compounds[a].

Microorganism	Extract 1	Extract 2	MG	IG	GP	Streptomycin (nystatin*)
Bacillus subtilis ATCC 6051	0.12	0.19	0.27	0.19	0.19	0.0052
Listeria monocytogenes ATCC 15313	0.31	0.31	0.31	0.22	0.27	0.016
Micrococcus flavus ATCC 10786	0.31	0.22	0.22	0.22	0.27	0.0052
Micrococcus luteus ATCC 10240	0.15	0.19	0.20	0.15	0.31	0.016
Proteus mirabilis ATCC 14273	0.12	0.19	0.20	0.19	0.19	0.0052
Sarcina lutea ATCC 10054	0.27	0.19	0.31	0.31	0.22	0.038
Staphylococcus aureus ATCC 25932	0.15	0.22	0.31	0.22	0.15	0.0052
Staphylococcus epidermidis ATCC 12228	0.12	0.19	0.27	0.22	0.22	0.0052
Streptococcus faecalis ATCC 12952	0.27	0.22	0.22	0.19	0.19	0.027
Escherichia coli ATCC 25922	0.15	0.19	0.20	0.15	0.12	0.0052
Enterobacter cloacae ATCC 13883	0.22	0.27	0.27	0.31	0.19	0.038
Pseudomonas aeruginosa ATCC 27853	0.12	0.27	0.27	0.15	0.22	0.016
Pseudomonas tolaasii NCTC 387	0.27	0.27	0.27	0.31	0.22	0.027
Salmonella typhimurium ATCC 14028	0.15	0.19	0.22	0.19	0.15	0.038
Salmonella enteritidis ATCC 13076	0.19	0.15	0.22	0.19	0.19	0.016
Candida albicans ATCC 10231	0.12	0.22	0.31	0.27	0.27	0.0052*

[a] Extract 1, methanolic extract of *G. lutea* leaves; extract 2, methanolic extract of *G. lutea* flowers; MG, mangiferin; IG, isogentisin; GP, gentiopicrin.

erin against several bacterial species has been reported previously (Stoilova *et al.*, 2005).

Our study indicated that each tested compound did not possess a dominant role in the antimicrobial activity of crude extracts. Thus, synergistic activity may be responsible for the inhibitory effect of the extracts. Since crude extracts of *G. lutea* leaves and flowers showed a wide range of antimicrobial effect, their use in the treatment of various bacterial and fungal infections could be beneficial.

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