

SHORT COMMUNICATION

Antibacterial Activity of Flavonoids and Phenylpropanoids from *Marrubium globosum* ssp. *libanoticum*

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Marrubium globosum Montbr. et Auch. ex Benth. ssp. *libanoticum* Boiss. (Lamiaceae) is a medicinal plant used for the treatment of inflammatory diseases, asthma, coughs and other pulmonary and urinary problems. The goal of our study was to assess the biological activity of *M. globosum* testing the methanol extract of aerial parts for its antibacterial activity against bacteria known to cause respiratory, gastrointestinal, skin and urinary disorders; the extract showed antibacterial effects against all the strains of bacteria used. A purification of this active extract showed the presence, as main constituents, of verbascoside, isorhamnetin 3-*O*- β -D-rutinoside, quercetin 3-*O*- β -D-rutinoside, naringenin 7-*O*- β -D-glucoside, kaempferol 3-*O*- β -D-rutinoside, isorhamnetin 3-*O*- β -D-glucoside, quercetin 3-*O*- β -D-glucoside, apigenin 7-*O*-(3''-*p*-coumaryl)-glucoside, *p*-methoxy-cinnamic acid, kaempferol 3-*O*- β -D-glucoside and apigenin 7-*O*- β -D-glucoside. The pure compounds were tested for their antibacterial activity; quercetin 3-*O*- β -D-rutinoside, verbascoside and naringenin 7-*O*- β -D-glucoside showed the greatest activity. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: *Marrubium globosum* ssp. *libanoticum*; Lamiaceae; flavonoid; phenylpropanoid; antibacterial activity.

INTRODUCTION

Aqueous and hydroalcohol extracts of flowered aerial parts of different *Marrubium* sp. are reported in the literature to be used in European folk medicine for treating cough and as a choleric in digestive and biliary complaints (Wichtl and Anton, 1999; Newall *et al.*, 1996); moreover, they are used in traditional medicine for their neurosedative and antiinflammatory activities (Mascolo *et al.*, 1987; Girre, 2000). Previous studies conducted on a hydroalcohol extract of *Marrubium vulgare* have demonstrated that it exerts a significant and non-specific antispasmodic effect on isolated smooth muscle (Schlemper *et al.*, 1996). The same extract exhibited potent and dose-dependent antinociceptive action in several models of pain in mice (De Souza *et al.*, 1998). Treatment with an aqueous extract of the aerial parts of *M. vulgare* significantly lowered the systolic blood pressure in spontaneously hypertensive rats (SHR), related to a vasodilatory effect exhibited by the extract *ex vivo* as well as *in vitro* (El Bardai *et al.*, 2001). Aqueous or hydroalcohol extracts of flowered aerial parts of *M. globosum* ssp. *libanoticum*, called 'hashiashat el kelb' in Northern Lebanon, are used in folk medicine. Decoctions or infusions of flowers and leaves are com-

monly used as hypoglycaemics (diabetes), febrifuges (malaria), antispasmodics (colics), for urinary tract infections and have external applications against snake bites and as cicatrizants of wounds (Wichtl and Anton, 1999). Recently, our research group has thoroughly studied the plant (Grassia *et al.*, 2006; Rigano *et al.*, 2006a, 2006b, 2006c) showing among other things that the acetone extract of this species exerts antiinflammatory effects in rat paw oedema induced by carrageenin (Rigano *et al.*, 2006b).

Some of the illnesses and complaints against which *Marrubium* is traditionally used are due to infectious noxes; antimicrobial properties of the plant were therefore assumed. Thus, the aim of this study was to investigate the methanol extract of *M. globosum* ssp. *libanoticum* and its constituents for their antimicrobial activities, in order to validate the ethnopharmacological uses of the plant.

MATERIALS AND METHODS

Collection of plant material. *M. globosum* aerial parts were collected from flowering plants in August 2002 on Col de Cèdres (Lebanon), at 2340 m above sea level. The identification was done by Professor N. A. Arnold, University of Saint Esprit, Lebanon, and confirmed by Professor T. Raus, Botanischer Garten, Berlin. A voucher specimen (NAP # 23) is deposited at the Herbarium Neapolitanum (NAP), Dipartimento

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Preparation of the methanol extract and isolation of compounds. Dry plant inflorescences (250 g) were minced into small pieces and then sequentially extracted by cold maceration with solvents of increasing polarity [petrol ether (40–60°) (3 × 2.5 L), CHCl₃ (3 × 2.5 L) and CH₃OH (3 × 2.5 L)]. The methanol solution was concentrated under reduced pressure, to yield 30 g of methanol extract (yield 12% w/w with respect to the dried powder). Compounds **1–11**, verbascoside (**1**, 50.5 mg, 0.2% yield), isorhamnetin 3-*O*-β-D-rutinoside (**2**, 23.2 mg, 0.09% yield), quercetin 3-*O*-β-D-rutinoside (**3**, 29.2 mg, 0.12% yield), naringenin 7-*O*-β-D-glucoside (**4**, 5.1 mg, 0.02% yield), kaempferol 3-*O*-β-D-rutinoside (**5**, 25.9 mg, 0.1% yield), isorhamnetin 3-*O*-β-D-glucoside (**6**, 17.1 mg, 0.07% yield), quercetin 3-*O*-β-D-glucoside (**7**, 2.5 mg, 0.01% yield), apigenin 7-*O*-(3''-*p*-coumaryl)-glucoside (**8**, 11.2 mg, 0.04% yield), *p*-methoxy-cinnamic acid (**9**, 44.6 mg, 0.18% yield), kaempferol 3-*O*-β-D-glucoside (**10**, 9.2 mg, 0.04% yield) and apigenin 7-*O*-β-D-glucoside (**11**, 28.3 mg, 0.11% yield) were isolated as described previously (Rigano *et al.*, 2006a).

Test organisms. Standard bacterial strains, obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA), were employed. They included Gram-positive (G+) bacteria: *Staphylococcus epidermidis* (ATCC 10875), *Staphylococcus aureus* (ATCC 13709), *Enterococcus faecalis* (ATCC 14428) and *Bacillus subtilis* (ATCC 10774) and the following Gram-negative (G-) bacteria: *Proteus mirabilis* (ATCC 7002), *Proteus vulgaris* (ATCC 12454), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 19430), *Enterobacter aerogenes* (ATCC 13048), *Enterobacter cloacae* (ATCC 10699), *Klebsiella pneumoniae* (ATCC 27736) and *Escherichia coli* (ATCC 11229).

MIC determination. The methanol extract and compounds **1–11** were added with 5 × 10⁻² M stock solution in DMSO. They were diluted from 0.01 to 1000 µg/mL

concentrations in sterile physiological Tris buffer (pH 7.4, 0.05 M) (Ieven *et al.*, 1979) immediately before being used.

Bacterial strains were grown on MH agar plates (Difco, Detroit, MI) and suspended in MH broth (Difco). The MIC values against bacterial strains were performed using the Ericsson and Sherris (1971) broth-dilution method (MH broth). The inoculum suspensions were prepared from 6 h broth cultures and adjusted to obtain a 0.5 McFarland standard turbidity. The plant material was sterilized by filtration through Millipore filters (0.45 µm) and added to MH broth medium. Serial 10-fold dilutions were made for a concentration range of 0.01–1000 µg/mL for the plant material. In the range between the minimum active and the maximum inactive concentrations, 2-fold dilutions of the extract and the metabolites were tested to obtain a more precise measure of the MIC. The bacterial suspensions were aerobically incubated for 24 h at 37 °C. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. Cultures containing only sterile physiological Tris buffer (pH 7.4, 0.05 M), which did not influence bacterial growth, were used as controls. The MIC values were also determined for tetracycline hydrochloride (Pharmacia, Milano), benzyl penicillin sodium (Cynamid, Catania) and cefotaxime sodium (Roussel Pharma, Milano) in MH broth using standard method.

Each compound was tested in triplicate, the experiment was performed four times.

RESULTS AND DISCUSSION

The methanol extract of *M. globosum* showed a strong inhibition against all the bacterial strains tested (MIC between 8 and 128 µg/mL, Table 1), even the particularly resistant *P. aeruginosa* and *S. aureus* were inhibited; among these, *S. epidermidis* showed the lowest MIC (8 µg/mL), while less sensitive to the extract was *B. subtilis*, with a MIC of 128 µg/mL. With the aim of isolating and characterizing the active compounds in

Table 1. Antibacterial activity of *M. globosum* methanol extract and of metabolites isolated from it^a

	Extract	1	2	3	4	6	7	9	11	Cefotax.	Penicil.	Tetrac.
Bacteria												
G(+)												
<i>Enterococcus faecalis</i> (ATCC 14428)	16	32	R	8	64	64	32	256	R	R	2	2
<i>Staphylococcus epidermidis</i> (ATCC 10875)	8	32	R	8	64	32	16	256	R	0.1	0.03	0.1
<i>Staphylococcus aureus</i> (ATCC 13709)	16	16	R	32	128	32	16	128	R	2	0.03	2
<i>Bacillus subtilis</i> (ATCC 10774)	128	128	R	128	256	256	R	R	256	32	32	2
G(-)												
<i>Enterobacter aerogenes</i> (ATCC 13048)	32	16	R	128	64	128	128	256	256	R	R	4
<i>Enterobacter cloacae</i> (ATCC 10699)	32	32	R	128	128	256	128	256	256	R	R	4
<i>Escherichia coli</i> (ATCC 11229)	32	32	256	64	64	128	32	256	R	0.1	64	4
<i>Klebsiella pneumoniae</i> (ATCC 27736)	16	R	R	64	32	32	16	R	R	0.1	R	16
<i>Proteus mirabilis</i> (ATCC 7002)	32	32	R	16	64	64	32	R	R	0.03	4	32
<i>Proteus vulgaris</i> (ATCC 12454)	64	4	R	64	64	128	64	R	R	0.03	R	4
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	32	32	256	64	64	128	64	128	R	1.6	R	32
<i>Salmonella typhi</i> (ATCC 19430)	64	64	R	64	128	128	64	256	R	0.5	4	1

^a Values represent MIC values expressed in µg/mL. A comparison is shown between the antibiotic activity of the plant extract with reference antibiotics: cefotax., Na-cefotaxime; penicil., benzyl penicillin sodium; tetrac., tetracycline. R, absence of inhibition even at the highest concentration used (1000 µg/mL).

M. globosum, the methanol extract was subjected to a chromatographic purification by gel filtration and HPLC to give, as major constituents, compounds **1–11**. As can be seen in Table 1, the highest antibacterial activity was shown by quercetin 3-*O*- β -D-rutinoside (**3**); all the bacteria were affected, especially *S. epidermidis* and *E. faecalis* (MIC = 8 μ g/mL). High antibacterial efficacy was shown also by verbascoside (**1**): all the strains, with the exception of *K. pneumoniae*, were inhibited, especially *P. vulgaris* (MIC = 4 μ g/mL) and its observed antimicrobial activity is in agreement with literature data (Calis *et al.*, 2005). Other active compounds were naringenin 7-*O*- β -D-glucoside (**4**), that showed activity against all the bacteria used with MIC between 32 and 256 μ g/mL, and quercetin 3-*O*- β -D-glucoside (**7**), active against all the bacteria (MIC between 16 and 128 μ g/mL) except *B. subtilis*. As can be seen in Table 1, (**3**) and (**7**) activities and spectra were similar, showing that the aglyconic moiety is crucial for activity. Isorhamnetin 3-*O*- β -D-rutinoside (**2**), isorhamnetin 3-*O*- β -D-glucoside (**6**), *p*-methoxy-cinnamic acid (**9**) and apigenin 7-*O*- β -D-glucoside (**11**) led only to a weak inhibition of micro-organism growth; as for kaempferol 3-*O*- β -D-rutinoside (**5**), apigenin 7-*O*-(3''-*p*-coumaryl)-glucoside (**8**) and kaempferol 3-*O*- β -D-glucoside (**10**) there was a total

absence of inhibition for all the concentrations used.

In the past years flavonoids have become the subject of anti-infective research, and many groups have isolated and identified the structures of this class of natural products possessing antibacterial activity (Cushnie and Lamb, 2005). Moreover, several researchers have showed the synergy between active flavonoids; for instance Kimura and Yamada (1984) have demonstrated that an association among quercetin, rutin and isorhamnetin-3-rutinoside, all present in the extract of *M. globosum* ssp. *libanoticum*, has an antimicrobial activity stronger than the single components.

In conclusion, some of the secondary metabolites isolated from *M. globosum* ssp. *libanoticum* showed an interesting antibacterial activity, which may explain the use of this species in folk medicine to treat skin and urinary tract infections as well as gastrointestinal diseases.

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