

POLYPHENOLIC COMPOUNDS OF *SIDERITIS LYCIA* AND THEIR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Four phenylpropanoid glycosides lavandulifolioside **1**, martynoside **2**, verbascoside (=acteoside) **3** and leucosceptoside A **4**, were isolated from aerial parts of *Sideritis lycia* Boiss. et Heldr. (Lamiaceae). Their structures were determined by chromatographic and spectroscopic methods (UV, IR, ¹H-NMR, ¹³C-NMR, FAB-MS). These compounds and flavonoid glycosides isolated from the same plant in our previously study were screened for their anti-inflammatory activity using the carrageenan-induced mouse paw edema test. The active compounds were then investigated for their effect on gastric ulceration. Although flavonoid glycosides showed higher activity than the phenylpropanoid glycosides, the gastric ulceration effect of phenylpropanoid glycosides was less than flavonoid glycosides.

INTRODUCTION

Sideritis species are widely distributed in Turkey and infusions of some *Sideritis* species have been used in the treatment of gastrointestinal disorders, common cold and as a diuretic as well as herbal tea in folk medicine (Baytop, 1984). In our previous studies, diuretic, anti-inflammatory (Yeşilada & Ezer, 1989), antispasmodic (Ezer et al., 1992), antibacterial (Ezer et al.,

1994) and antimicrobial (Akçoş et al., 1998) activities of different extracts of some *Sideritis* species grown in Mediterranean region were confirmed. The components of the essential oil and flavonoid glycosides **5-6** of an endemic species *Sideritis lycia* Boiss. et Heldr. have also been determined (Ezer et al., 1995; Ezer & Akçoş, 1995). However, in *S. lycia*, neither the confirmation of the presence of these compounds nor their activity has been determined before. Therefore, in this study we aimed to isolate the phenylpropanoids of *S. lycia* and screen their activity. Four phenylpropanoid glycosides were isolated and their structures were identified as lavandulifolioside **1**, martynoside **2**, verbascoside (acteoside) **3** and leucosceptoside A **4**. Since *Sideritis* species have been reported to contain compounds with anti-inflammatory activity (Villar et al., 1984; Gabrieli et al., 1990), we first investigated the anti-inflammatory activities of the petroleum ether, chloroform, ethyl acetate and aqueous extracts of *S. lycia*. Ethyl acetate and aqueous extracts showed high anti-inflammatory activity. Further investigation revealed significant anti-inflammatory activity of phenylpropanoid glycosides isolated from the ethyl acetate and aqueous extract and flavonoid glycosides isolated from ethyl acetate extract, with a low incidence of gastric ulceration.

Keywords: *Sideritis lycia*, Lamiaceae, phenylpropanoid glycosides, flavonoid glycosides, anti-inflammatory activity, gastric ulceration

MATERIALS AND METHODS

Plant Material

The aerial parts of *Sideritis lycia* Boiss. et Heldr. were collected from Antalya-Kemer, around the Göynük stream in July 1992. A voucher specimen has been deposited at the Herbarium of the Faculty of

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Apparatus and Materials

IR and UV spectra were recorded on a Perkin-Elmer 457 and Shimadzu UV-160 A, respectively. ¹H- and ¹³C-NMR including DEPT spectra were recorded on a Bruker WM-300 NMR spectrometer. Positive ion FAB-MS spectra was determined on a Kratos AEI-MS 50 mass spectrometer. MPLC was performed with RP-C18 Sepalyte 40 μm (column dimensions 352 × 18 mm). For TLC, silica gel 60F₂₅₄, plates (Merck 5554) were applied. Polyamide (Macherey-Nagel SC₆ < 0.05–0.16 mm), silica gel 60 (0.063–0.2 mm, Merck 7734) and Sephadex LH-20 were used for column chromatography. For the anti-inflammatory activity and gastric ulceration, carrageenan and indomethacin (Sigma) were used.

Extraction

Powdered aerial parts (1100 g) were extracted with methanol (3 × 3 l). The combined extracts were evaporated under vacuum to dryness. The residue was dissolved in water and the water-soluble part was extracted with petroleum ether, chloroform and ethyl acetate, respectively.

Isolation

Compound **1** (30 mg) was isolated from the aqueous phase (20 g) using polyamide column chromatography with water/methanol (75:25) to (25:75) as solvent system and Sephadex LH-20 using water as eluent.

Ethyl acetate extract (7.30 g) was first chromatographed over polyamide eluting with water, followed by increasing concentrations of methanol. The fractions found to be rich in phenylpropanoid glycosides were combined and rechromatographed over silica gel eluting with chloroform/methanol/water (70:30:3). Two fractions containing compounds **2-4** were obtained. Fr. A was chromatographed over silica gel eluting with chloroform/methanol (90:10) yielded **2** (11.5 mg) which was further purified over Sephadex LH-20 using water as eluent. Fr. B was applied to MPLC using an eluent containing increasing amounts of methanol in water as solvent system to afford compounds **3** (220 mg) and **4** (6 mg).

Anti-Inflammatory Activity

Animals: Male local bred mice 20 ± 2 g body weight, were obtained from the Department of Experimental Animals, Hacettepe University.

Carrageenan-induced mouse paw edema (CPE) was measured using a Peacock dial thickness gauge (0.01–10 mm). Six mice per group were used. Sixty minutes after i.p. administration of crude extracts (1.5 g/kg) (Yeşilada & Ezer, 1989), active compounds (100 mg/kg) (Şafak et al., 1992) and 10 μl of 2% carrageenan were injected subcutaneously in 0.9% saline solution (w/v) into the plantar surface of the right hind paw. One hour later the volume of edema was measured with a dial thickness gauge (Şafak et al., 1992).

Ethyl acetate and aqueous extracts and active compounds were homogenized in 0.9% saline solution (w/v), and petroleum ether and chloroform extract were homogenized in dimethylsulfoxide. Control group animals were injected with either 0.9% saline solution (w/v) or dimethylsulfoxide.

Gastric Ulceration Studies

Mice were fasted for 24 h (with water *ad libitum*). Four hours after i.p. administration of compounds, animals were sacrificed and the stomachs were examined for lesions under dissecting microscope (Sancilio et al., 1987).

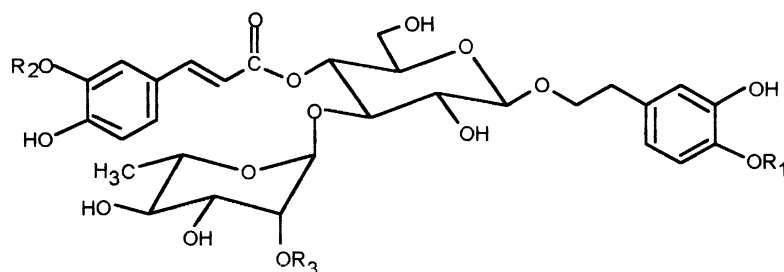
Statistical Analysis

Student's *t*-test and analysis variance (ANOVA) were used (Bliss & White, 1967).

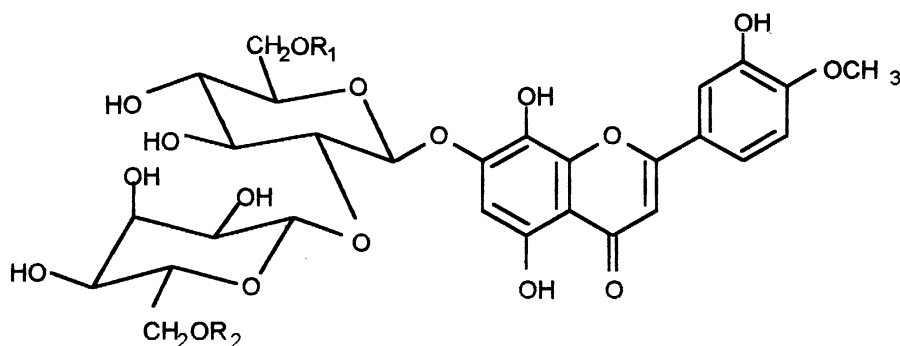
RESULTS AND DISCUSSION

Lavandulifolioside [**1**]

UV λ_{max}^{MeOH} (nm): 332.4, (288.8), (247.8), (219.8), 204.2. IR [KBr, cm⁻¹ (T%)] 3400, 1699, 1620, 1600, 1525, 1449, 1276. ¹H-NMR (300 MHz, CD₃OD, TMS int): δ (ppm) = 6.70 (d, *J* = 1.7 Hz, H-2), 6.70 (d, *J* = 8.0 Hz, H-5), 6.60 (dd, *J* = 8.0/1.7 Hz, H-6), 4.08/3.80–3.70 (m, H-a), 2.80 (t, *J* = 7.2 Hz, H-b), 4.41 (d, *J* = 7.9 Hz, H-1'), 5.53 (br.s, H-1''), 4.35 (d, *J* = 7.1 Hz, H-1'''), 7.09 (d, *J* = 1.8 Hz, H-2'''), 6.80 (d, *J* = 8.2 Hz, H-5'''), 6.99 (dd, *J* = 8.2/1.8 Hz, H-6'''), 6.31 (d, *J* = 15.9 Hz, H-a'), 7.63 (d, *J* = 15.9 Hz, H-b'). ¹³C-NMR (75 MHz, CD₃OD, TMS int): δ (ppm) = 131.5 (C-1), 117.0 (C-2), 146.1 (C-3), 144.7 (C-4), 117.1 (C-5), 121.3 (C-6), 71.9 (C-a), 36.6 (C-b), 104.2 (C-1'), 102.0 (C-1''), 107.5 (C-1'''), 127.6 (C-1'''), 114.7 (C-2'''), 146.8 (C-3'''), 149.8 (C-4'''), 116.4 (C-5'''), 123.2 (C-6'''), 115.2 (C-a'), 148.0 (C-b'), 168.3 (C=O). FAB-MS, *m/z* (rel. int) [%]: 779 (4.4) [M+Na]⁺, 625 (1.7) [M+H-Arabinose]⁺.



	R₁	R₂	R₃	
1	1	H	H	-Arabinose
2	2	-CH ₃	-CH ₃	H
3	3	H	H	H
4	4	H	-CH ₃	H



	R₁	R₂	
5	5	H	-COCH ₃
6	6	-COCH ₃	-COCH ₃

Martynoside [2]

UV $\lambda_{\text{max}}^{\text{MeOH}}$ (nm): 328.2, (284.2), (233.2), (220.2), 205.0.
 IR [KBr, cm^{-1} (T%)] 3400, 1730, 1620, 1600, 1516, 1456 1275. ¹H-NMR (200 MHz, CD₃OD, TMS int): δ (ppm)= 6.84 (d, $J = 2.2$ Hz, H-2), 6.75 (dd, $J = 2.2$ Hz, H-6), 4.01–3.40(H-a), 2.91 (t, $J = 7.24$ Hz, H-b), 3.90 (s, -OCH₃, H-4), 4.47 (d, $J = 7.98$ Hz, H-1'), 5.28 (d, H-1''), 7.29 (d, $J = 1.74$ Hz, H-2'''), 7.14 (dd, $J = 1.74$ Hz, H-6'''), 6.47 (d, $J = 15.9$ Hz, H-a'), 7.75 (d, $J = 15.9$ Hz, H-b'), 3.96 (s, -OCH₃, H-3''').

Verbascoside [3]

UV $\lambda_{\text{max}}^{\text{MeOH}}$ (nm): 332.6, (288.8), (248.8), (219.8), 204.4.
 IR [KBr, cm^{-1} (T%)] 3390, 1698, 1620, 1600, 1525, 1448, 1283. ¹H-NMR (200 MHz, CD₃OD, TMS int): δ (ppm)= 6.78 (d, H-2), 6.76 (d, $J = 8.06$ Hz, H-5), 6.65 (dd, $J = 8.06$ Hz, H-6), 4.04/3.72–3.69 (m, H-a), 2.87

(t, $J = 7.2$ Hz, H-b), 4.47 (d, $J = 7.9$ Hz, H-1'), 5.27 (d, $J = 1.6$ Hz, H-1''), 7.14 (d, $J = 1.88$ Hz, H-2'''), 6.86 (d, $J = 8.14$ Hz, H-5'''), 6.06 (dd, $J = 8.14/1.88$ Hz, H-6'''), 6.36 (d, $J = 16.08$ Hz, H-a'), 7.68 (d, $J = 16.08$ Hz, H-b').

Leucosceptoside A [4]

UV $\lambda_{\text{max}}^{\text{MeOH}}$ (nm): 372.2, (285.8), (235.4), (219.2), 204.0.
 IR [KBr, cm^{-1} (T%)] 3390, 1699, 1620, 1600, 1518, 1450, 1279. ¹H-NMR (200 MHz, CD₃OD, TMS int): δ (ppm)= 6.87 (d, $J = 1.92$ Hz, H-2), 6.78 (d, $J = 8.04$ Hz, H-5), 6.65 (dd, $J = 8.04/1.92$ Hz, H-6), 4.17–4.08/3.60 (m, $J = 2.2$ Hz, H-a), 2.88 (t, $J = 7.5$ Hz, H-b), 4.47 (d, $J = 7.82$ Hz, H-1'), 5.28 (d, $J = 1.7$ Hz, H-1''), 7.16 (d, $J = 2.1$ Hz, H-2'''), 7.18 (dd, $J = 8.1/2.1$ Hz, H-6'''), 6.42 (d, $J = 15.9$ Hz, H-a'), 7.71 (d, $J = 15.9$ Hz, H-b'), 3.98 (s, -OCH₃, H-3''').

Swelling (thickness, $\times 10^{-2}$ mm) \pm S.E. and inhibition rates (%) of the different extracts and isolated compounds were measured and the results are given in Tables 1 and 2a. Gastric ulceration results of the isolated compounds are given in Table 2b.

The structure of compound **1** was established on the basis of UV, IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and positive ion FAB-MS data, and the structure of compound **1** was identified as lavandulifolioside (Başaran et al., 1988).

Table 1. Effect of different extracts of *Sideritis lycia* on CPE test.

Extract	Dose(mg/kg) i.p.	1 h	2 h	3 h	4 h
Control	–	48.33 \pm 12.01	36.66 \pm 14.81	75.00 \pm 20.20	68.33 \pm 20.27
Petroleum ether	1500	51.66 \pm 12.01 (–)	21.66 \pm 14.81 (36.36)	23.33 \pm 8.33 (68.89)*	13.33 \pm 10.92 (80.49)
Chloroform	1500	46.66 \pm 6.66 (3.45)	8.33 \pm 6.0 (77.28)	15.00 \pm 2.88 (80.00)*	5.00 \pm 2.88 (92.68)
Ethyl acetate	1500	21.66 \pm 9.27 (55.18)	25.00 \pm 10.40 (31.80)	31.66 \pm 17.40 (57.78)*	31.66 \pm 13.01 (53.66)
Aqueous	1500	38.33 \pm 14.52 (20.69)	28.33 \pm 8.33 (22.72)	23.33 \pm 1.6 (68.89)*	43.33 \pm 3.30 (36.58)
Indomethacin	10	44.16 \pm 12.20 (18.50)	52.00 \pm 10.40 (11.80)	43.00 \pm 10.20 (44.00)*	48.00 \pm 11.70 (47.00)

n = 6, *p < 0.05

Swelling (thickness, $\times 10^{-2}$ mm) \pm SE and inhibition rates compared to control (% in parenthesis).

Table 2a. Effect of phenylpropanoid glycosides and flavonoid glycosides of *Sideritis lycia* on CPE test.

Drug	Dose(mg/kg) i.p.	1 h	2 h	3 h	4 h
Control	–	54.16 \pm 3.00	59.16 \pm 3.00	77.50 \pm 3.8	92.50 \pm 8.92
[1]	100	71.66 \pm 8.33 (–)	71.66 \pm 8.33 (–)	73.33 \pm 12.01 (5.19)	71.66 \pm 15.89 (22.82)
[2] + [4]	100	65.00 \pm 8.36 (–)	70.00 \pm 14.81 (–)	73.33 \pm 8.02 (5.00)**	80.00 \pm 6.8 (13.00)
[3]	100	54.16 \pm 3.00 (9.09)	55.00 \pm 0.002 (8.33)	68.33 \pm 8.33 (12.82)*	100.00 \pm 7.63 (1.57)
[5] + [6]	100	88.33 \pm 7.14 (–)	76.66 \pm 6.0 (–)	67.50 \pm 5.43 (12.90)*	80.00 \pm 6.95 (13.00)
Indomethacin	10	44.16 \pm 12.20 (18.50)	52.00 \pm 10.40 (11.80)	43.00 \pm 10.20 (44.00)*	48.00 \pm 11.70 (47.00)

n = 6, *p < 0.05, **p < 0.01

Swelling (thickness, $\times 10^{-2}$ mm) \pm SE and inhibition rates compared to control (% in parenthesis).

[1]: Lavandulifolioside, [2]: Martynoside, [3]: Verbascoside, [4]: Leucosceptoside A, [5]: 4-O-Methyl ether of Hypolaetin 7-O-[6'''-O-acetyl- β -D-allopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside], [6]: 4'-O-Methyl ether of Hypolaetin 7-O-[6'''-O-acetyl- β -D-allopyranosyl-(1 \rightarrow 2)-6''-O-acetyl- β -D-glucopyranoside].

Table 2b. Gastric ulceration results of the phenylpropanoid glycosides and flavonoid glycosides of *Sideritis lycia*.

Drug	Ulcer incidence (No./group)	Lesion length (mm \pm S.E.)
[1]	6/6	0.03 \pm 0.0004
[2] + [4]	3/6	0.30 \pm 0.083
[3]	0/6	–
[5] + [6]	4/6	0.13 \pm 0.040
Indomethacin	6/6	0.20 \pm 0.080

n = 6, SE: Standard error.

[1]: Lavandulifolioside, [2]: Martynoside, [3]: Verbascoside, [4]: Leucosceptoside A, [5]: 4-O-Methyl ether of Hypolaetin 7-O-[6'''-O-acetyl- β -D-allopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside], [6]: 4'-O-Methyl ether of Hypolaetin 7-O-[6'''-O-acetyl- β -D-allopyranosyl-(1 \rightarrow 2)-6''-O-acetyl- β -D-glucopyranoside].

The UV, IR and ¹H-NMR spectral data for the compounds **2-4** are in good accordance with those reported for martynoside (Çalış et al., 1988), verbascoside (Andary et al., 1982; Çalış et al., 1988) and leucosceptoside A (Çalış et al., 1988), respectively.

Verbascoside, which is widespread in the Lamiaceae, was isolated from *Sideritis perfoliata* previously (Ezer et al., 1992). Martinoside, leucosceptoside A and lavandulifolioside have been isolated for the first time from *S. lycia* in this study.

Maximum edema is achieved by the third hour in the CPE test (Vinegar et al., 1969; Villar et al., 1986). In initial activity screening, extracts were active in the CPE test (Table 1). Since *Sideritis lycia* has been used in infusions forms in traditional medicine, we planned to screen the activities of phenylpropanoid glycosides (**1-4**) and flavonoids (**5-6**) isolated from ethyl acetate and remaining aqueous extracts. Although flavonoid glycosides **5+6** (Ezer & Akcoş, 1995) showed higher activity than the phenylpropanoid glycosides **1-4** (Table 2a), the gastric ulceration effect of phenylpropanoid glycosides less than flavonoid glycosides (Table 2b). We then performed gastric ulceration test **2+4** and **3** were promising compounds with 50% less gastric ulceration than indomethacin. We further investigated compound **3** since gastric ulceration with this compound was nil (0). ED₅₀ value for compound **3** was 62.5 mg/kg.

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