Caffeoyl Quinic and Tartaric Acids and Flavonoids from *Lapsana communis* L. subsp. *communis* (*Asteraceae*)

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Six hydroxycinnamic acids : caffeic acid, chlorogenic acid, 3,5-O-dicaffeoylquinic acid 2-O-caffeoyltartaric acid (caftaric acid) and 2,3-O-dicaffeoyltartaric acid (chicoric acid) have been isolated from *Lapsana communis* L. *subsp. communis* aerial parts. Among flavonoids, only isoquercitrin, luteolin and luteolin-7-O-β-glucuronide were identified. Except for chlorogenic acid, these compounds represent the first report in *Lapsana communis*. Chicoric acid is the major phenylpropanoic constituent in this plant.

Introduction

Lapsana communis L. *subsp. communis* is an *Asteraceae* included in the tribe of *Lactuceae* and currently placed into the subtribe *Crepidinae* (Pak and Bremer, 1995). Its vernacular name "Nipplewort" is due to the use of the plant in folk medicine up to the twenty century. Fresh juice of stems or fresh leaves were applied as a poultice against chapped nipples or hands (Cazin, 1868). Leaves and stems are reported in France to possess diuretic and hypoglycemic activities (Leclerc, 1954). Nipplewort was also eaten in salad, like dandelion (Couplan, 1992), but today this herb has fallen into oblivion.

The polyphenol constituents of *Lapsana communis* have been the object of only a few research studies. The total amount of polyphenols in aerial parts amounts to 5.5% (Carr and Bagby 1987). Among them, chlorogenic acid (Bandyukova *et al.*, 1970a) and luteolin-7-O-β-D-glucoside (Bandyukova *et al.*, 1970b) were identified in epigeal parts. In the present study, we report the polyphenolic compounds composition of *L. communis subsp. communis* samples collected in Touraine (France). The investigation was carried out on aerial parts (leaves, stems, flowers) and on the roots.

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Results and Discussion

Eight phenolic compounds were detected in the aqueous EtOH or $(Me)_2CO$ extracts of aerial parts of *L. communis subsp. communis*. The structures and occurrence of these compounds are given in Table I.

Compound **1** was characterized as caffeic acid according to TLC, ^{1}H NMR and ^{13}C NMR spectrum by comparaison with an authentic sample. The ^{1}H NMR spectrums of compounds **2** and **3** exhibit signals belonging to caffeoyl and quinoyl moieties. The signals of H-3, H-4 and H-5 of the quinoyl moiety were assigned according to their shift and coupling constants. The structure of **2** was identified as chlorogenic acid and compound **3** as 3,5-O-dicaffeoylquinic acid. Assignments are consistent with data previously reported (Cheminat *et al.*, 1988; Abdel Sattar *et al.*, 1995).

Compounds **4** and **5** are tartaroyl esters of caffeic acid. The compound **4** was identified as 2,3-O-dicaffeoyltartaric acid (chicoric acid) according to TLC, ^{1}H NMR, ^{13}C NMR and FAB-MS (négative ions) *m/z*: 473 $[M-H]^-$ (Becker and Hsieh, 1985; Veit *et al.*,1991). The ^{1}H NMR spectrum of **5** was carried out in CD_3OD, D_2O and then acetone-d_6 to avoid water signal present in CD_3OD or D_2O solvents. Spectrum of **5** in acetone-d_6 exhibits signals belonging to a caffeoyl moity: three aromatic protons (6.90–7.21 ppm), two *trans*-vinyl protons (6.34, 7.68 ppm, J = 15.8 Hz). Two other signals H-3 (4.85 ppm, d, J = 2.6 Hz) and H-2 (5.60 ppm, d, J = 2.6 Hz) are present. ^{1}H NMR, FAB-MS (negative ions) *m/z*: 311 $[M-H]^-$, 446 $[M+triethanolamine-H_2O]^-$, UV, TLC and alkaline hydrolysis indicated that **5** is 2-O-caffeoyltartaric acid (caftaric acid) according with earlier reports (Soicke *et al.*, 1988; Biau S., 1996).

Among flavonoids only three were isolated in significant amounts in *L. communis subsp. communis*. The structures of luteolin **(6)** and isoquerci-

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Table I. Comparaison of the caffeic acid derivatives and flavonoid constituents of *Lapsana communis subsp. communis* tissues.

		Plant		Tissue	
	Compound	Root	Stem	Leaf	Flower
1	Caffeic acid	tr	+	+	+
2	Chlorogenic acid	+	+	+	+
3	3,5-O-dicaffeoylquinic acid	–	–	+	+
4	Chicoric acid	–	tr	++	+
5	Caftaric acid	tr	tr	+	+
6	Luteolin	–	–	tr	++
7	Luteolin-7-O-β-D-glucuronide	–	+	+	+
8	Isoquercitrin	–	tr	tr	+

tr., traces.

trin **(8)** were determined by spectroscopic methods (UV, ^1H and ^{13}C NMR, COSY, FAB$^-$-MS) and direct comparison with authentic samples. Compound **7** was identified as luteolin-7-O-β-D-glucuronide on the basis of its R_f value, acid and enzymatic hydrolysis, ^1H NMR, FAB-MS, and UV spectra (Darbour *et al.*, 1996). It gave an UV spectrum nearly equal to that of the luteolin except that the lack of any shift in band II with NaOAc indicated a 7-O-substituted structure (Markham, 1982). Acid and enzymatic hydrolysis gave luteolin and glucuronic acid.

A comparison of hydroxycinnamic acids and flavonoïds constituents between different tissues of *L. communis subsp. communis* was realised with TLC densitometry. Results are presented in Table I. All hydroxycinnamic acids detected in this herb have been previously reported in other *Asteraceae* (Molgaard and Ravn, 1988). Among them chicoric acid was found to be the major constituent in the aerial part of *L. communis*.

The flowers are especialy rich in luteolin as in the case of dendelion (Williams *et al.*, 1996). Like other genera of the same subtribe, *L. communis* contains the widespread luteolin-7-O-glucuronide (Terencio *et al.*, 1993), but luteolin-7-O-glucoside was not detected during our study in spite of previous published data on epigeal parts of *Lapsana communis* (Bandyukova *et al.*, 1970b).

Materials and Methods

TLC was performed using silica gel 60 G (Merck Germany) with EtOAc:HOAc:H_2O (8:2.2:2 v/v)(eluant 1) and for aglycones with toluene:EtOAc:HCOOH (5:4:1 v/v)(eluant 2). The caffeoyl derivatives were characterised as blue spots by UV irradiation (365 nm) and, with flavonoids, by spraying with Neu reagent followed UV irradiation (365 nm). TLC densitometry was carried out on a TLC scanner 76510 (Camag), beam sizes : 0.4 × 1.2 mm, spotted volumes : 20 µl with Linomat 4 (Camag), UV detections were performed at 330 and 400 nm. Sephadex LH-20 (Pharmacia) was used for CC. ^1H and ^{13}C NMR spectra were recorded at 200 MHz and 50 MHz respectively. FAB-MS were performed on a Vacuum Generator (V.G) TRIO 2 mass spectrometer, 70 eV, and a mixture of glycerol and triethanolamine (1:1 v/v) or thioglycerol as matrix.

Plant material

Lapsana communis L. *subsp. communis* was collected at the flowering stage in Notre Dame d'Oé (Indre et Loire, France) during July 1995. Voucher specimens was deposited in the Herbarium of the Laboratory of Pharmacognosy (Faculty of Pharmaceutical Sciences, Tours) (N° 51).

Extraction and isolation

Dried and finely powdered aerial parts of plant material (70 g) were extracted with EtOH:H_2O (1:3 v/v) (700 ml) at room temp. for 24 hr. EtOH was then evaporated (45 °C, under reduced pressure). The aqueous solution acidifed to pH 2 with 5% H_2SO_4 was further extracted with Et_2O (4 × 100 ml). The concentrated Et_2O layer was finally subjected on columm chromatography with Sephadex LH-20 (2.5 × 20 cm) with H_2O/EtOH (100% up to 70:30 v/v) as eluant yielding caftaric acid

(5)(100:0 v/v) (4 mg), caffeic acid **(1)**(75:25 v/v) (15 mg), chicoric acid **(4)**(70:30 v/v) (30 mg). Dried and finely powdered aerial parts (70 g) were extracted with Me_2CO:H_2O (4:1 v/v) (700 ml) at room temp. for 24 hr. Me_2CO was evaporated. The aqueous solution was successively extracted with Et_2O and EtOAc. The concentrated EtOAc layer was fractionated over Sephadex LH-20 (2.5 × 20 cm) with eluant H_2O:EtOH (100% up to 60%) yielding chlorogenic acid **(2)**(20:80 v/v) (8 mg), luteolin-7-O-β-D-glucuronide **(7)**(25:75 v/v) (5 mg), isoquercitrin **(8)**(40:60 v/v) (13 mg), 3,5-O-dicaffeoylquinic acid **(3)**(60:40 v/v) (15 mg). Dried and semi-powdered flowers of plant (70 g) were extracted with EtOH:H_2O (1:3 v/v) (700 ml) at room temp. 24 hr. EtOH was evaporated. The aqueous solution was extracted with Et_2O (4 × 50 ml). Et_2O layer was concentrated to dryness and washed with CH_2Cl_2. The residue was dissolved in H_2O and let at 2 °C for 24 hr. giving a precipitate of pure luteolin **(6)** (26 mg).

5.0 g of each dried and pulverized tissue samples (flowers, leaves, steams and roots) were extracted with 50.0 ml of EtOH:H_2O (1:1 v/v) far 24 hours at room temp. and filtered before TLC densitometry determination. R_f values with eluant 1 : **(5)** 0.22, **(2)** 0.46, **(7)** 0.51, **(4)** 0.57, **(8)** 0.63, **(3)** 0.80, **(1, 6)** 0.95. R_f values with eluant 2 : **(6)** 0.55, **(1)** 0.60.

The structures of the eight isolated compounds were elucidated by TLC, UV using standard procedures, ^{1}H-NMR, alkaline hydrolysis for esters; acid and enzymatic hydrolysis for compounds **7**, **8**. ^{13}C NMR were performed for compounds **1**, **3**, **4**, **8**; COSY for compounds **3**, **8**; and MS-FAB$^-$ for **4**, **5**, **7**, **8**. Authentic samples of **1**, **2**, **6**, **8** were purchased from Extrasynthèse (Saint Quentin Fallavier, France).

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