Triterpene Alcohols and Fatty Acids in Lipids and Nonsaponifiable Matter of *Lapsana communis* L. Subspecies *communis* (Asteraceae)

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ABSTRACT: The oil content of samples of aerial parts of *Lampsana communis* L. subsp. *communis,* harvested in Indre et Loire (France) at different periods, varies from 1.1 to 2.1%. Nonsaponifiable matter in these samples represents 36.0 to 65.7% of the oil. Yields of triterpene alcohols of nonsaponifiable matter (25.9 to 81.8%) were determined by preparative thin-layer chromatography after saponification. Seven of them were identified by gas chromatography–mass spectrometry (GC–MS). Six of the fatty acids in the aerial parts have also been identified by GC–MS.The seeds of *L. communis* contain 2.6% oil.
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KEY WORDS: Asteraceae, fatty acids, Lactuceae, *Lapsana communis* L. subsp. *communis,* lipids, nonsaponifiable matter, triterpene alcohols.

Lapsana communis L. subsp. *communis* is a rustic herbaceous plant that belongs to the family of Asteraceae, the tribe of Lactuceae, and the subtribe of Crepidinae. Although fallen into oblivion and considered a weed, this plant was formerly used in popular medicine in several European countries to nurse cracked nipples (1). Its chemical composition is poorly known. Only three of its constituents, chlorogenic acid (2), luteoline-7-*O*-glucoside (3) and mannitol (4), have been described.

In the framework of a plant species valorization program, conducted since 1974 under the auspices of the United States Department of Agriculture (USDA), one report, published in 1987, indicated that the aerial parts of *L. communis* gave the highest yield of oil (6.1%) among 51 plants analyzed (5). Of nearly 1,000 species previously analyzed, only 30 have yielded a minimum of 6% oil with a maximum of about 11% (5). According to this same publication, *L. communis* contains the following lipid families: fatty acids, fatty alcohols, sterols, esters of triglycerides and triterpene alcohols, with nonsaponifiable matter and free fatty acid values of 12.6 and 69.5%, respectively (5).

Our research goal was to compare results published from *L. communis* collected in the United States with those obtained from French *L. communis* and to evaluate the most abundant lipids by seeking to identify those that could explain its reported therapeutic properties.

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MATERIALS AND METHODS

Equipment. Preparative thin-layer chromatography (TLC) of nonsaponifiable matter was carried out on Silica gel 60, 1 mm thickness (Merck, Darmstadt, Germany) with the eluent hexane/Et_2O (1:1 vol/vol). Mass spectra of trimethylsilylated triterpene alcohols and methylated fatty acids were obtained with a Hewlett-Packard (Palo Alto, CA) 5989 H mass spectrometer. Ion source temperature was 200°C, and quadrupole temperature was set at 100°C. The ionizing voltage was 70 eV. A Hewlett-Packard 5890 Series II gas chromatograph was equipped with an HP-5MS capillary column, 0.25 µ, 30 m × 0.25 mm i.d. The programmed temperature was increased 10°C/min to 260°C, then kept steady for 40 min. The helium carrier gas was set at 1 mL/min.

Plant material. Samples of aerial parts, leaves, and seeds of *L. communis* L. subsp. *communis* were collected at Notre Dame d'Oé (Indre et Loire, France). They were dried at 40°C.

Lipid extraction. The pulverized tissue sample (50 g) was extracted 2 × 8 h in a Soxhlet extractor with hexane. After cooling, kieselguhr was added to remove chlorophyll from the solution. After removing the kieselguhr, the solution was evaporated to dryness.

Isolation of nonsaponifiable matter and fatty acids. Lipids (1 g) and 50 mL of 2 N KOH in EtOH 96% vol/vol were boiled for 1 h. After cooling, 50 mL water was added. This solution was extracted with 3 × 50 mL hexane. The top layers were washed with 50 mL of 50% vol/vol EtOH, dried over Na_2SO_4, and evaporated to dryness to give the nonsaponifiable matter. The bottom layer was acidified to a pH of 1.5 with HCl, then extracted by 2 × 30 mL hexane. The hexane layers were washed with 50 mL water, dried over Na_2SO_4, and evaporated to dryness to give the fatty acids.

Methylation of fatty acids. Fatty acids (50 mg) were converted to methyl esters by treatment with 1.7 mL isopropyl ether and 0.4 mL of trimethylsulfonium hydroxide (Macherey-Nagel, Düren, Germany) 2 M in MeOH (6).

TABLE 1
Lipids, Nonsaponifiable Matter and Triterpene Alcohols from *Lapsana communis*

Tissue[a]	Month of harvest	Oil content (% w/w)	NM[b]/oil (% w/w)	TA[c]/NM[b] (% w/w)	TA[c]/oil (% w/w)
F.a.p.	August 1995	2.01	43.2	42.5	18.4
Young a.p.	October 1995	1.56	36.0	25.9	9.3
A.p.b.	May 1996	1.14	48.0	81.8	39.2
F.a.p.	June 1996	1.15	65.7	71.5	46.9
A.p.	August-September 1982 and 1983	6.1[d]	12.6[d]		

[a] F.a.p., dried flowered aerial parts; young a.p., dried "rosettes" from plants germinated at the beginning of September; a.p.b., dried aerial parts before blossom; a.p., dried aerial parts.
[b]NM, nonsaponifiable matter.
[c]TA, triterpene alcohols.
[d]Reported values from the literature (5).

Isolation and silylation of triterpene alcohols. The nonsaponifiable matter (40 mg) was separated by preparative TLC. The compounds with R_f 0.45 on silica gel, eluted by hexane/ether (1:1 vol/vol), gave the triterpene alcohol fraction. Ten milligrams of the triterpene alcohol fraction was derivatized in 200 µL of the following reagent: chlorotrimethylsilane/1,1,1,3,3,3-hexamethyldisilazane (both from Aldrich, Saint Quentin Fallavier, France)/anhydrous pyridine (1:1:9 vol/vol/vol) to give the trimethylsilyl (TMS) ether. After 30 min under vacuum, the solution was centrifuged.

RESULTS AND DISCUSSION.

Soxhlet extraction by hexane of dried aerial parts or dried leaves of *L. communis* gives a waxy and pale yellow residue at room temperature. It was necessary to confirm the oil yield of several samples because our results (Table 1) were low compared with values (6.1%) from a previous report (5). The previous experimental protocol (5) (extraction of the plant by acetone, and isolation of the oil in the top layer of hexane/87.5% ethanol) was tried but did not give a higher oil recovery. Changes in the raw material, leaves instead of aerial parts, harvest date, or increase in extraction times did not allow us to reach 6.1% oil recovery. Such differences may be explained by the origin of the plants and/or by the physiological stage at harvest. Important output differences were observed between samples harvested in August of 1995 and those harvested in May and June of 1996 in the same area. The 2.6 % oil content of the seeds was also low.

The fatty acids of oil from the aerial parts were identified by gas chromatography–mass spectrometry (GC–MS) (Table 2). All fatty acids were detected in the samples harvested in May and August.

In comparison to the previous data (5), the ratio of nonsaponifiable matter/oil of samples collected in Indre et Loire (France) is high (Table 1).

By TLC and spraying with vanillin sulfuric reagent, the chromatograms of nonsaponifiable matter, taken from all samples of *L. communis,* gave intense deep purple spots, which corresponded to the triterpene alcohols. Preparative TLC allowed us to isolate the triterpene alcohol fraction and determine the ratio to nonsaponifiable matter (Table 1).

Separation of triterpene alcohol components by GC–MS demonstrated the presence of at least 10 compounds, seven of which were identified on the basis of mass spectral evidence (Table 3). The mass spectral data of **2–7** and **9** are well known and identical to those of taraxerol, β-amyrin, germanicol, α-amyrin, lupeol, cycloartenol, and ψ-taraxasterol, respectively (7–9). Unknown compound **8** has been partially identified because of its resemblance to the spectrum of ψ-taraxasterol. The spectra are nearly the same except for three different fragments, 514 $[M]^+$, 499 $[M - Me]^+$, and 409 $[M - (CH_3 + TMSiOH)]^+$, which indicate a methyl group instead of a $D^{20(21)}$ double bond. GC–MS analysis alone was not sufficient to determine the position of the methyl group.

Helianol was not detected in *L. communis* in spite of its high frequency in Asteraceae (10).

In conclusion, lipids of *L. communis* collected in Indre et Loire (France) are characterized by an exceptional amount of triterpene alcohols. Recently, it has been demonstrated that many of these compounds possess anti-inflammatory properties (11–14). The large amount of triterpene alcohols in *L. communis* could explain therapeutic effects on the skin or nipples, crack healing, and reduction in the inflammation of nipples according to folk usage (1).

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TABLE 2
GC–MS Data of Fatty Acids from *L. communis*[a]

Fatty acids	Rrt	Mass spectrum (70 eV), *m/z* (relative intensity) of $[M]^+$
Myristic acid	1.08	242 (4)
Palmitic acid	1.23	270 (10)
10,13- or 11,14-Octadecadienoic acid	1.34	294 (9)
α-Linolenic acid	1.35	292 (9)
Stearic acid	1.36	298 (9)
Arachidic acid	1.48	326 (6)

[a]Compounds are arranged in the elution order on GC. Relative retention times (Rrt) are expressed relative to lauric acid methyl ester. Data for fatty acids correspond to those for the methyl ester derivatives. GC–MS, gas chromatography–mass spectrometry. For other abbreviation see Table 1.

TABLE 3
GC–MS Data and Mass Spectra of Triterpene Alchols from *L. communis*[a]

Triterpene alcohols	Rrt	Mass spectrum (70 eV), *m/z* (relative intensity)
Unidentified (**1**)	1.36	692 (3), 602 (6), 411 (8), 339 (9), 283 (10), 207 (13), 205 (9), 189 (20), 124 (37), 69 (100)
Tarax-14-en-3β-ol (taraxerol) (**2**)	1.45	498 (1) $[M]^+$, 374 (4), 359 (5), 269 (6), 218 (15), 204 (42), 189 (10), 73 (100)
Olean-12-en-3β-ol (β-amyrin) (**3**)	1.50	498 (2) $[M]^+$, 393 (1), 359 (1), 279 (2), 218 (100), 203 (34), 189 (20)
Olean-18-en-3β-ol (germanicol) (**4**)	1.52	498 (3) $[M]^+$, 484 (2), 232 (7), 218 (10), 205 (9), 204 (33), 190 (16), 189 (31), 177 (26), 95 (32), 73 (100)
Urs-12-en-3β-ol (α-amyrin) (**5**)	1.57	498 (2) $[M]^+$, 279 (4), 218 (100), 203 (16), 189 (23)
Lup-20(29)-en-3β-ol (lupeol) (**6**)	1.60	498 (4) $[M]^+$, 408 (2), 393 (2), 279 (4), 369 (6), 306 (1), 299 (1), 279 (4), 231 (6), 218 (12), 203 (10), 189 (21), 95 (35), 73 (100)
9β,19-cyclolanost-24-en-3β-ol (cycloartenol) (**7**)	1.61	498 (2) $[M]^+$, 408 (4), 393 (8), 365 (4), 189 (6), 175 (6), 95 (25), 75 (66), 69 (100)
Unidentified (**8**)	1.68	445 (3), 405 (3), 331 (4), 292 (11), 229 (13), 218 (12), 203 (16), 95 (42), 73 (100)
Unidentified (**9**)	1.77	514 (2) $[M]^+$ 499 (4), 483 (2), 416 (1), 409 (3), 408 (3), 393 (3), 369 (4), 279 (5), 218 (4), 204 (7), 203 (6), 189 (44), 73 (100)
Taraxast-20-en-3β-ol (ψ-taraxasterol) (**10**)	1.80	498 (3) $[M]^+$, 483 (1), 416 (1), 408 (5), 393 (2), 369 (5), 279 (5), 218 (5), 204 (6), 203 (7), 190 (17), 189 (33), 73 (100)

[a]Compounds are arranged in the elution order on GC. Rrt are expressed relative to trimethylsilylsitosterol. Data for triterpene alcohols correspond to those for the trimethylsilyl derivatives. For abbreviations see Tables 1 and 2.

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