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Analytical Discrimination of Poisonous and Nonpoisonous Chemotypes of Giant Fennel (*Ferula communis* L.) through Their Biologically Active and Volatile Fractions

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Giant fennel (*Ferula communis* L.) from Sardinia is characterized by two chemotypes with different biological activities. One chemotype is poisonous, due to prenylcoumarins, and responsible for ferulosis, which mainly affects sheep and goats, cattle, and horses; the other chemotype is nonpoisonous and contains daucane esters. The two chemotypes cannot be distinguished botanically. High-performance liquid chromatography−diode array−ultraviolet detection−mass spectrometry (HPLC-DAD-UV-MS) analysis of the composition of the fractions containing the biologically active metabolites and of the volatile fractions, by gas chromatography−mass spectrometry (GC-MS), of both essential oil and headspace sampled by headspace solid-phase microextraction (HS-SPME) are here shown to be effective in discriminating the poisonous and nonpoisonous chemotypes. HS-SPME with CAR/PDMS/DVB in combination with GC-MS has also been found to be a successful, fully automated one-step method for rapid and unequivocal discrimination of the two chemotypes, using aristolene and allohedycaryol as markers of the poisonous and nonpoisonous chemotypes, respectively.

KEYWORDS: ***Ferula communis* L.; poisonous chemotype; nonpoisonous chemotype; prenylated coumarins; daucane esters; essential oils; headspace**

INTRODUCTION

The *Ferula* genus (Apiaceae) has long been of considerable interest because of its biological activity. It consists of about 130 species growing wild from the Mediterranean areas to Central Asia. Ferulae are characterized by lattices and gum resins or oleoresins, widely used in traditional medicines to treat various diseases, for example, asafoetida (*F. assa-foetida*), galbanum (*F. galbaniflua*), and ammoniacum africanum (*F. communis* var. *brevifolia*) (*1*).

Ferula communis L., also known as giant fennel, is widespread (probably the most widespread species of this genus) in Mediterranean areas and southern Saudi Arabia and includes several varieties, the botanical classifications of which have not yet been fully clarified (*2*). *F. communis* grows wild throughout Sardinia (Italy) and is known to include two chemotypes, which cannot be distinguished botanically despite having totally different biological activities, one chemotype being highly toxic and the other chemotype nontoxic (*1*, *3*). **Figure 1** shows the distribution of the two *F. communis* chemotypes in Sardinia (*4*). The toxic variety is thought to be responsible for a lethal hemorrhagic syndrome known as "ferulosis", which mainly affects sheep and goats, cattle, and horses (*1*, *5*).

The toxicity is due to two quite rare groups of coumarin C-prenylated derivatives with different basic skeletons: 4-hydroxycoumarin and pyrane-[3,2-*C*]-coumarin, in particular, ferulenol (**1**) and ferprenine (**3**) (**Figure 2**) (*1*, *6*, *7*). On the other hand, the nontoxic Sardinian variety is mainly known for its not scientifically proved aphrodisiac activity and, to a lesser extent, for its antibacterial properties, due to the estrogenic properties of a group of esters of sesquiterpenic alcohols, with a daucane skeleton, mainly derived from ferutinol (also known as jaeschkeanadiol) (**4**) and siol (**8**), with aromatic acids, the most abundant of which are ferutinin (**5**), teferin (**6**), and teferdin (**7**) (*1* and references cited therein).

Unfortunately, the two chemotypes do not show any morphological differences and have the same somatic chromosome number ($2n = 24$); therefore, other approaches must be used to

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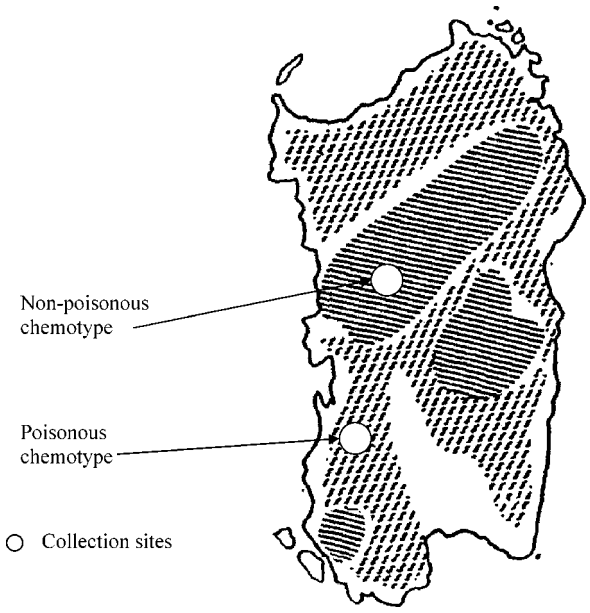

Figure 1. Chemotype distribution of *F. communis* in Sardinia (*4*).

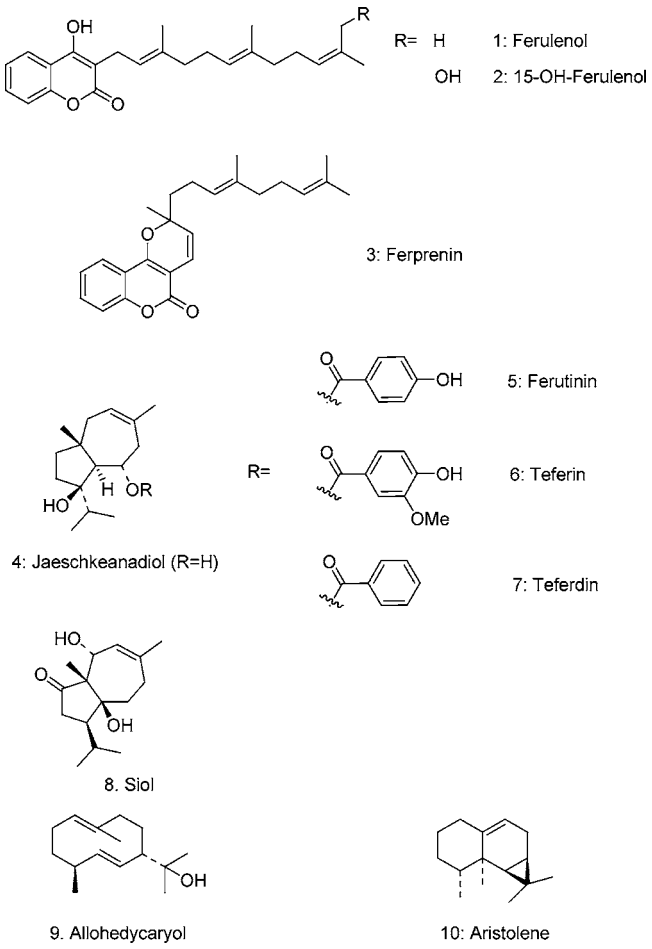

Figure 2. Secondary metabolites characterizing *F. communis* L. chemotypes.

distinguish between them. Sacchetti et al. (*8*) described two methods to discriminate the poisonous from the nonpoisonous chemotype: the first based on thin layer chromatography (TLC) analysis of the latex diluted 10-fold in ethyl acetate and using ferulenol and ferutinin as markers, and the second based on fluorescence microscopy of the fresh vittae using incident ultraviolet (UV) light at 365 and 436 nm. Both methods were applied to 115 samples of *F. communis* collected throughout Sardinia to build a "ferulosis" risk map of the island (**Figure 1**). Arnoldi et al. (*9*) recently described a method based on methanol-sonicated extraction of dried roots combined with high-performance liquid chromatography−diode array−ultraviolet−mass spectrometry (HPLC-DAD-UV-MS); appropriate fingerprints to discriminate between the two chemotypes were obtained, and 5 prenylcoumarins and 11 daucane esters were identified. Surprisingly, the nontoxic chemotype was also found to contain ferulenol (**1**), in addition to ferutinin (**5**), as its main component.

This study on the chemical composition of the fraction containing the biologically active metabolites, as well as that of the volatile fraction, was undertaken to discriminate the poisonous from the nonpoisonous chemotypes of giant fennel (*F. communis* L.). Moreover, to achieve better insight on this species, distribution of the biologically active components of the two chemotypes in the different parts of the plant was also investigated. The volatile fraction compositions of the two Sardinian chemotypes were investigated to find markers suitable to distinguish between them, as well as to develop a reliable, one-step, easy-to-automate analysis method to replace the conventional time-consuming solvent extraction combined with HPLC-DAD-UV-MS.

MATERIALS AND METHODS

Plant Material and Chemicals. Three samples of leaves, latex, stems, and roots from different plants belonging to each *F. communis* chemotype from different parts of Sardinia (Iglesias for poisonous chemotype and Seneghe for nonpoisonous chemotype) were collected in early spring of 2003 and 2004. Pure standards of ferutinin, teferin, teferdin, ferulenol, 15-hydroxyferulenol, and jaeschkeanadiol were kindly provided by Prof. G. Appendino (DISCAFF, Università del Piemonte Orientale, Novara, Italy). Voucher specimens (no. 612 for the poisonous chemotype and no. 612/b for the nonpoisonous one) are preserved for reference in the Department of Botany, University of Cagliari (Italy). HPLC and analytical grade solvents from Carlo Erba Reagenti, Rodano, Italy, were used throughout.

Sample Preparation. *Extraction of Biologically Active Fractions. Plant Material.* Five hundred milligrams of leaves, stems, and roots of both chemotypes was sonicated for 10 min with acetonitrile (8 mL) three times. The resulting total extract (24 mL) was filtered, reduced to 10 mL by evaporation under vacuum, and extracted with *n*-hexane (10 mL). The remaining acetonitrile phase was then evaporated to dryness under vacuum; the weighed solid residue was rediluted in acetonitrile at a concentration of 1 mg/mL and analyzed by HPLC-DAD-UV and high-performance liquid chromatography−mass spectrometer detector (HPLC-MSD).

Latex. Half a millliter of latex was extracted with acetone (15 mL) three times. The resulting extracts were combined (45 mL) and evaporated to dryness under vacuum. The solid residue was then diluted with acetonitrile (10 mL) and extracted with *n*-hexane (10 mL), and the acetonitrile phase was evaporated to dryness under vacuum; the weighed solid residue was rediluted in acetonitrile at a concentration of 1 mg/mL and analyzed by HPLC-DAD-UV and HPLC-MSD.

Volatile Fraction. Essential Oil Preparation. Essential oil was prepared in agreement with the *European Pharmacopoeia* (*10*). Three hundred grams of fresh crushed plant was suspended in 3.0 L of water in a 6.0 L reactor for 1 h and then submitted to hydrodistillation in a modified Clevenger apparatus for 4 h. The resulting essential oil was left to stabilize for 1 h. Hydrodistillation was repeated six times for a total of 2 kg of processed plant material, obtaining about 200 μL of essential oils from the poisonous chemotype (yield = 0.01%) and 1 mL from the nonpoisonous one (yield = 0.05%).

Headspace Solid-Phase Microextraction (HS-SPME) Sample Preparation. The SPME device and fibers were purchased from Supelco Co. (Bellefonte, PA). Several fibers of polydimethylsiloxane (PDMS) 100 μm, PDMS/polydivinylbenzene (DVB) 65 μm, Carboxen (CAR)/PDMS

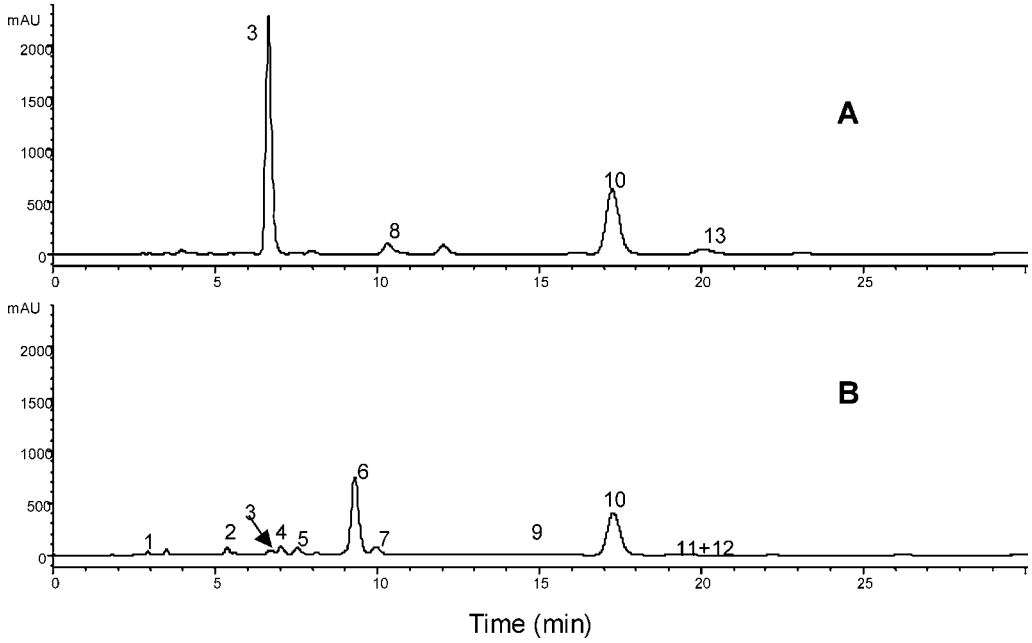

Figure 3. HPLC-DAD-UV profiles at 210 nm of the latex extracts of both (**A**) poisonous and (**B**) nonpoisonous chemotypes. Peaks: 1, 2-hydroxyferutidin; 2, acetoxyferutinin; 3, 15-hydroxyferulenol; 4, lapiferin; 5, MW 388; 6, ferutinin; 7, teferin; 8, acetoxyferulenol; 9, akiferin; 10, ferulenol; 11 + 12, teferdin + ferutidin; 13, benzoyloxyferulenol.

75 μm, and a three-component fiber CAR/PDMS/DVB (2 cm long, with a coating volume of 1.000 mm^3) were tested. Before use, all fibers were conditioned as recommended by the manufacturer.

Plant material (200 mg) hermetically sealed in a 2.0 mL vial was introduced in a thermostatic bath at 80 °C (25 °C in the case of latex). After 15 min, the SPME device was inserted in the sealed vial containing the sample, and the CAR/PDMS/DVB fiber was exposed to the matrix headspace (sampling time = 30 min). The vial was vibrated for 10 s every 5 min with an electric engraver (Vibro-Graver V74), (Burgess Vibrocrafters Inc., Brayslake, IL). After sampling, the SPME device was immediately inserted into the GC injector and the fiber thermally desorbed. A desorption time of 5 min at 230 °C was used. Before sampling, each fiber was reconditioned for 20 min in the GC injection port at 230 °C.

HPLC-DAD-UV Analysis. HPLC-DAD-UV analyses were carried out on a 1050 series HP system provided with an Agilent 1100 diode array detector (Agilent Technologies, Waldbronn, Germany). A 250 × 4.6 mm i.d., 5 μm, Nucleosil C18 100-5 column (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was used. Analysis conditions were as follows: mobile phase, eluent A, acetonitrile with 0.1% phosphoric acid; eluent B, water with 0.1% phosphoric acid; mobile phase gradient, 75% A for 25 min, then 100% A for 30 min; injection volume, 20 μL; flow rate, 1 mL/min; UV detection wavelengths, 210 and 230 nm.

Quantitative Analysis. Suitable amounts of ferutinin, teferin, teferdin, ferulenol, and 15-hydroxyferulenol were diluted with acetonitrile to obtain concentrations corresponding, respectively, to 1, 0.5, 0.1, 0.05, and 0.01 mg/mL of each marker. Standard solutions containing 2 mg/mL 15-hydroxyferulenol and a 0.001 mg/mL ferulenol were also prepared. A calibration curve was made by analyzing the resulting standard solutions three times by HPLC-DAD-UV at 210 nm.

HPLC-MSD Analysis. HPLC-MSD analyses were carried out with a single-quadrupole HP-1100 D MSD system (Agilent Technologies) equipped with an orthogonal atmospheric pressure chemical ionization (APCI) source. The same column as for HPLC-DAD-UV analysis was used. Mobile phase and mobile phase gradient were the same as for HPLC-DAD-UV, but phosphoric acid was replaced by formic acid; flow rate = 0.3 mL/min. APCI-MSD conditions: nebulizer pressure, 35 psi; nebulizer temperature, 350 °C; vapor flow, 6 L/min; vaporizer temperature, 300 °C; corona, 5 μA; capillary voltage, 4000 V; fragmentation, 70 eV; mass range, 100−500 Da.

Analysis conditions were optimized by analyzing pure standards of ferutinin, teferin, teferdin, ferulenol, and 15-hydroxyferulenol, directly in flow injection.

GC and GC-MS Analysis. GC analyses were carried out on a Thermo Electron Trace GC Ultra device provided with a high-frequency fast FID detector (300 Hz, time constant = 6 ms). Data processing was by Hyper Chrom software (version 2.3) (Thermo Electron Rodano, Italy). Capillary GC-MS analyses were performed on an Agilent 6890 GC-5973N MS system (Little Falls, DE). The injection volume was 1 μL of essential oil diluted 1:200 in cyclohexane.

GC analysis conditions were as follows: injection temperature, 250 °C; mode, split; split ratio, 1:20; detector temperature, 270 °C; columns, 25 m × 0.25 mm i.d., 0.25 μm, OV-1 FSOT polydimethylsiloxane and 25 m × 0.25 mm i.d., 0.3 μm, polyethyleneglycol (PEG20M) MegaWax [Mega, Legnano (Milan), Italy]; temperature program, from 50 °C (1 min) to 220 °C (5 min) at 3 °C/min; carrier gas, hydrogen; flow rate, 1.0 mL/min in constant flow mode.

GC-MS analyses were carried out under the same conditions reported for GC except that helium was used as carrier gas: flow rate, 1.0 mL/min, in constant flow mode. MS was in EI mode at 70 eV. Ion source temperature was 230 °C.

Essential oil and headspace components were identified by comparison of both their linear retention indices, calculated versus a C_8−C_{25} hydrocarbon mixture, and their mass spectra with those of authentic samples or with data in the literature.

RESULTS AND DISCUSSION

The chemical composition of both the fraction containing the biologically active metabolites and the volatile fraction of three samples of each chemotype of giant fennel collected in different parts of Sardinia was studied with the aim of discriminating the poisonous from the nonpoisonous chemotype.

Biologically Active Fraction. The fractions containing prenylcoumarins and daucane esters, extracted from leaves, stems, roots, and latex by sonication with acetonitrile and cleaned up with *n*-hexane, were analyzed by HPLC-DAD-UV and by HPLC-APCI-single-quadrupole MS system (MSD). **Figure 3** shows the HPLC-DAD-UV profiles at 210 nm of the latex extracts of both poisonous (**A**) and nonpoisonous (**B**) chemotypes, whereas **Figure 4** shows the HPLC-APCI-MSD profiles

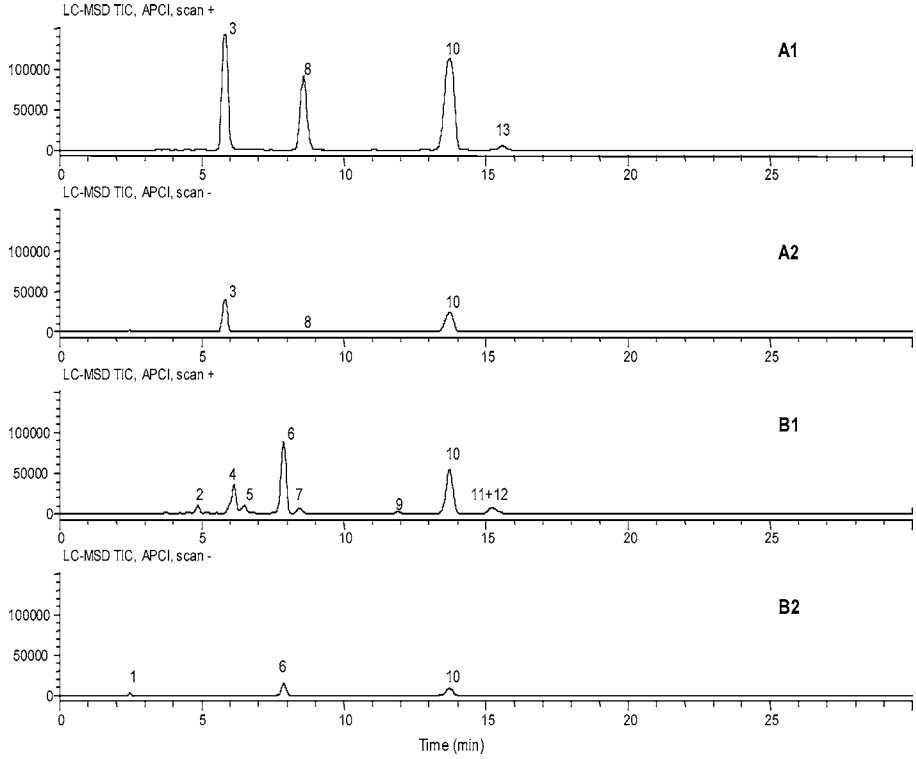

Figure 4. HPLC-APCI-MSD profiles in both positive (**A1**, **B1**) and negative mode (**A2**, **B2**) of the root extracts of poisonous (**A**) and nonpoisonous (**B**) chemotypes. For peak identification see **Figure 3**.

Table 1. Diagnostic Ions in Positive/Negative APCI Mode and UV Maxima of the Components Detected in HPLC-DAD-UV-MSD and the Proposed Attribution[a]

no.	compound	MW	APCI$^+$ (*m/z*)	APCI$^-$ (*m/z*)	UV_{max} (nm)
1	2-hydroxyferutidin	388		387 $[M - H]^-$	257
2	acetoxyferutinin	416	201 $[M - H_2O - AcOH - pOHBenzOH + H]^+$ 399 $[M - H_2O + H]^+$		257
3	15-hydroxyferulenol	382	365 $[M - H_2O + H]^+$, 383 $[M + H]^+$	381 $[M - H]^-$	202, 282, 307
4	lapiferin	394	217 $[M - H_2O - AngOH - AcOH + H]^+$ 395 $[M+H]^+$		218
6	ferutinin	358	341 $[M - H_2O + H]^+$	357 $[M - H]^-$	257
7	teferin	388	291, 371 $[M - H_2O + H]^+$	387 $[M - H]^-$	218, 262, 292
8	acetoxyferulenol	424	365 $[M - AcOH + H]^+$	363 $[M - H - AcOH]^-$	202, 282, 307
9	akiferin	402	385 $[M - H_2O + H]^+$		218, 262, 292
10	ferulenol	366	367 $[M + H]^+$	365 $[M - H]^-$	202, 282, 307
11 + **12**	teferdin + ferutidin	342, 372	325 $[M - H_2O + H]^+$ 355 $[M - H_2O + H]^+$		257
13	benzoyloxyferulenol	486	365 $[M - BenzOH + H]^+$		202, 282, 307

[a] Base peaks are underscored.

in positive (**A1**, **B1**) and negative modes (**A2**, **B2**) of the root extracts of poisonous and nonpoisonous chemotypes. **Table 1** lists the components identified by HPLC-APCI-MS in the two *F. communis* chemotypes, together with their diagnostic ions obtained in both positive and negative APCI modes and UV absorption maxima. The APCI interface was chosen to produce quasi-molecular or diagnostic ions that could easily be correlated to the analyte molecular mass. The components of the two chemotypes were identified by comparing their HPLC retention times, UV absorption maxima, and positive and negative mode APCI-MS fragmentation patterns to those of authentic samples (when available) and/or to the corresponding data reported by Arnoldi et al. (*9*). The latter authors adopted a different ionization approach and an ion-trap MS system, but the complementary use of HPLC elution sequence, UV spectra, and MS diagnostic ions could provide an effective analyte comparison for reliable identification. Moreover, with positive APCI-MS mode, daucane esters could easily be distinguished also through the medium-to-low-intensity ions diagnostic of the sesquiterpene skeleton at *m/z* 203 $(M - H_2O - acidOH + H)^+$ and 221 (i.e., $M - acidOH + H)^+$.

Quantitative analyses were carried out on the basis of the calibration curves obtained by analyzing standard solutions prepared with suitable amounts of ferutinin, teferin, teferdin, ferulenol, and 15-hydroxyferulenol. Linear regression equations, correlation coefficients, limits of detection (LOD), and limits of quantitation (LOQ) for each component mentioned above are reported in the Supporting Information.

Table 2 reports the quantitative distribution, in different parts of the plant, of the five components characteristic of the poisonous and nonpoisonous chemotypes and selected as markers. As already reported by Arnoldi et al. (*9*), in this case, too, 15-hydroxyferulenol and ferulenol (i.e., prenylcoumarins) were also found in the nonpoisonous chemotype, although in

Table 2. Percentage of Marker Compounds in the Different Parts of the Plant of *F. communis* L. Chemotypes

	leaves		stems		roots		latex	
compound	toxic	nontoxic	toxic	nontoxic	toxic	nontoxic	toxic	nontoxic
15-hydroxyferulenol (**2**)	0.05	0.01	0.50	0.01	4.19	0.07	1.44	0.03
ferutinin (**5**)		0.75		0.20		0.87		0.34
teferin (**6**)		0.06		0.01		0.02		0.02
ferulenol (**1**)	0.04	0.04	0.11	0.01	1.03	0.004	0.26	0.17
teferdin (**7**)		0.04		0.01		0.09		0.01

decidedly lower amounts than in the poisonous chemotype. On the contrary, daucane derivatives were not detected at all in the poisonous chemotype. In the poisonous samples, the highest amounts of 15-hydroxyferulenol and ferulenol were found in roots and latex (about 5 and 1%, respectively). The marker abundance in stems and leaves was considerably lower, because 15-hydroxyferulenol accounted for about 0.50% in stems and 0.05% in leaves and ferulenol for 0.10% in stems and 0.04% in leaves.

In the nonpoisonous samples, prenylcoumarins were found to be present in far smaller amounts. 15-Hydroxyferulenol accounted for about 0.03% in latex, 0.07% in roots, 0.005% in stems, and 0.01% in leaves, whereas ferulenol accounted for 0.004% in roots, 0.01% in stems, and 0.04% in leaves; the only exception being ferulenol in latex, which was about 0.2%. The most abundant daucane derivative was ferutinin, ranging from 0.87% in roots and 0.75% in leaves to 0.20% in stems. Teferin was present in percentages >10 times lower: in this case, too, the highest content was in latex, where it amounted to 0.025 and 0.014%, respectively. Traces of ferutidin coeluted with teferin. Repeatability of the results was very good because the highest RSD% was about 10% for ferulenol.

Volatile Fraction. The composition of the volatile fraction was also investigated, with the aim of determining whether the poisonous chemotype can be distinguished from the nonpoisonous one through components other than the biologically active prenylcoumarins and daucane derivatives. In this part of this work, volatile marker compounds, suitable to discriminate between the two chemotypes, have been searched and the possibility to develop an easy-to-automate method was evaluated. A number of studies have investigated *Ferula* species essential oils, in particular, *F. flabelliloba* (*11*), *F. stenocarpa* (*12*), *F. galbaniflua* (*13*), *F. gummosa* (*14*), *F. assa-foetida* (*15*), *F. microcolea* and *F. hirtella* (*16*), *F. persica* (*17*, *18*) from Iran, *F. elaeochytris* (*19*) from Turkey, *F. ferulaoides* (*20*) from Mongolia, and *F. arrigonii* (*21*) from Corsica (France). The essential oil composition of leaves, flowers, and peduncles of poisonous *F. communis* from Corsica, as well as its evolution during the development of the plant, was recently investigated by Tomi et al. (*22*), who found myrcene (53.5%), aristolene (5.3%), and farnesol (4.3%) as characterizing essential oil components. Very recently, Marongiu et al. (*23*) reported a comparison between the composition of the essential oil and the supercritical fluid extraction (SFE) of *F. communis;* in both extracts they found α- and β-gurjunene as main components. The present study investigated the essential oil and headspace composition of both chemotypes of *F. communis* from Sardinia. To the best of our knowledge, this is also the first comparison of the composition of the volatile fractions of the two Sardinian chemotypes of this species.

Essential Oils. The aerial parts of three samples of each chemotype of *F. communis* from different botanical stations in Sardinia were distilled, and the resulting essential oil was analyzed by GC-MS. **Table 3** lists components identified in both chemotypes, together with their linear retention indices on both OV-1 and PEG20M columns and area percent abundances. Although the plant material of both chemotypes was odorless, the two essential oils smelled differently; the one from the poisonous chemotype had a faint hydrocarbon smell, whereas that from the nonpoisonous chemotype had a decidedly more intense fresh, herbaceous, and slightly pungent smell.

Table 3. Components Identified in the Essential Oils of both *F. communis* L. Chemotypes

no.	compound	retention index on HP-5	retention index on CW20	*F. communis* poisonous chemotype (%)	*F. communis* nonpoisonous chemotype (%)
1	α-pinene	940	1030	0.6	3.3
2	β-pinene	971	1117		0.2
3	β-myrcene	995	1174	0.1	0.1
4	*p*-cymene	1030	1262	tr[a]	
5	limonene	1030	1207	0.1	0.1
6	*cis*-β-ocimene	1041	1225		0.1
7	*trans*-β-ocimene	1051	1251		tr
8	γ-terpinene	1060	1257	0.1	tr
9	α-terpinolene	1088	1292	tr	tr
10	linalool	1099	1565	0.5	0.2
11	fenchol	1113	1573	0.1	
12	terpinen-4-ol	1178	1619	tr	tr
13	α-terpineol	1190	1714	tr	tr
14	fenchyl acetate	1220	1464	0.3	
15	nerol	1230	1814	tr	
16	geraniol	1264	1856	tr	
17	α-copaene	1376	1483	0.1	0.1
18	daucene	1379	1495		0.1
19	β-elemene	1391	1584	tr	tr
20	aristolene	1417	1552	47.1	
21	aristola-1(10)-8 diene	1426	1576	2.0	
22	β-gurjunene	1430	1577	7.6	
23	*trans*-α-bergamotene	1438	1581	tr	tr
24	*trans*-β-farnesene	1459	1668		1.1
25	β-selinene	1484	1698	2.3	2.2
26	bicyclogermacrene	1495	1728		2.9
27	β-bisabolene	1509	1730		0.9
28	δ-cadinene	1523	1746	1.4	4.1
29	(*E*)-nerolidol	1565	2046	0.3	
30	caryophyllene oxide	1581	1965	1.3	
31	allohedycaryol	1590	2037		53.7
32	10-*epi*-γ-eudesmol	1619	2090		0.6
33	τ-muurolol	1643	2182		2.0
34	β-eudesmol	1649	2188		0.7
35	α-cadinol	1656	2196		4.2
36	MW 222 (base peak *m/z* 84)	1693	2282		12.1
37	daucane derivative	1700			
38	(*E*,*E*)-farnesol	1724	2345	21.2	
39	jaeschkeanadiol	1768			2.2

[a] Traces, below 0.1%.

These results clearly show that the essential oils can successfully be used to discriminate between the poisonous and nonpoisonous chemotypes from Sardinia. Both essential oils are characterized by very rich sesquiterpenoid fractions, although with completely different compositions. The essential oil from the poisonous chemotype is characterized by aristolene (47.1%) as the main component and farnesol (21.2%), whereas myrcene was identified as only a trace, unlike in the essential oils from

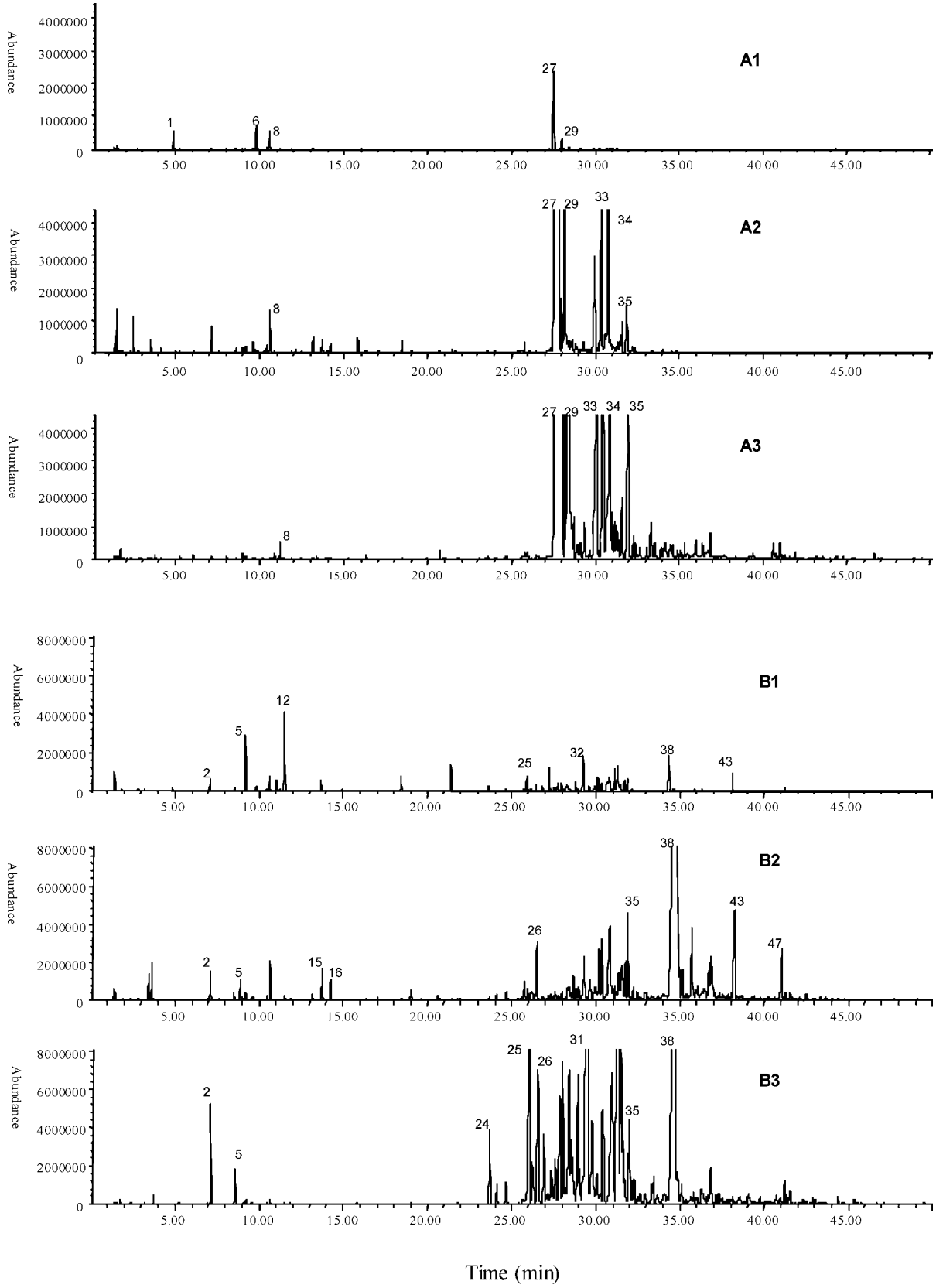

Figure 5. HS-SPME-GC-MS profiles of aerial parts (**A1**, **B1**), latex (**A2**, **B2**), and roots (**A3**, **B3**) of *F. communis* (**A**) poisonous and (**B**) nonpoisonous chemotypes. For peak identification see **Table 4**.

Corsica (*22*). On the other hand, the essential oil from the nonpoisonous chemotype is characterized by allohedycaryol as the main component (53%), an unidentified sesquiterpene alcohol (12%), and α-cadinol (4.2%). It is interesting to note the presence of daucene (0.1%) and jaeschkeanadiol (2.2%) in the nonpoisonous chemotype, that is, sesquiterpenoids having the same skeleton as that of the esters characterizing the biologically active fraction. The microdistillation (*24*) of a sample of latex (2 mL) from both chemotypes suspended in water did not produce a sufficient amount of essential oil to be

Table 4. Components Identified and Related Percent Abundance in the Headspace of *F. communis* L. Poisonous and Nonpoisonous Chemotypes by HS-SPME-GC-MS

		F. communis poisonous chemotype (%)			*F. communis* nonpoisonous chemotype (%)		
no.	compound	leaves	roots	latex	leaves	roots	latex
1	*cis*-3-hexenol	6.6			0.4		
2	α-pinene	0.6	tr[a]	0.3	1.4	1.2	0.6
3	benzaldehyde	0.4	tr				
4	β-pinene	0.3		tr	0.4	0.4	0.2
5	β-myrcene			tr	7.9	tr	0.1
6	*cis*-3-hexenyl acetate	10.7			0.5		
7	*p*-cymene	1.4		tr	0.2		
8	limonene	8.6	tr	0.3	2.2	0.1	1.0
9	benzyl alcohol		0.1				
10	*cis*-β-ocimene				1.5		
11	salicyl aldehyde		0.2	0.1			
12	*trans*-β-ocimene				12.6		tr
13	γ-terpinene	0.7		tr	0.2		
14	α-terpinolene	0.3		0.2			
15	linalool		tr	tr	1.5		0.9
16	fenchol			0.1			0.6
17	terpinen-4-ol			tr			
18	α-terpineol			tr			
19	decanal			0.6	2.4		
20	fenchyl acetate			tr			0.3
21	nerol			tr			tr
22	linalyl acetate		0.1	tr			
23	geraniol			tr			0.1
24	α-copaene		0.2	0.3	0.4		0.6
25	daucene				2.0	5.0	0.4
26	β-elemene		0.2	tr	1.3	2.8	1.9
27	aristolene	49.6	49.9	61.1		1.1	
28	aristola-1(10)-8 diene	0.7	2.7	1.5			
29	β-gurjunene	7.0	13.8	13.0			
30	*trans*-α-bergamotene					3.1	
31	*trans*-β-farnesene				6.5	15.2	1.6
32	germacrene D				2.7	2.6	0.8
33	β-selinene	1.1	5.4	4.3	2.4	0.8	2.7
34	bicyclogermacrene			3.9		5.8	
35	δ-cadinene	0.8	3.1	1.6	3.4		3.7
36	(*E*)-nerolidol		tr				
37	caryophyllene oxide	1.3					
38	allohedycaryol		tr	tr	6.8	16.2	44.9
39	10-epi-γ-eudesmol						2.8
40	β-eudesmol		0.4	0.3			1.3
41	daucane derivative					0.8	1.3
42	α-cadinol		1.5	0.7			0.8
43	MW 222 (base peak *m*/*z* 84)				3.5		4.3
44	(*E*,*E*)-farnesol		0.1	1.8			
45	aristolone		0.2	0.1			
46	jaeschkeanadiol					0.1	2.3
47	(*E*,*E*)-farnesyl acetate			0.6			

[a] Traces, below 0.1%.

analyzed by GC or GC-MS, probably because of the small amount of latex available. An exhaustive study on the composition of both essential oils is under way (unpublished results). The differences in essential oil composition of the two chemotypes therefore make the volatile fraction a reliable marker to distinguish between them.

HS-SPME Sampling. One of the limitations of essential oil analysis is that the time it takes is comparable to that required to analyze the biologically active semivolatile fractions. On the other hand, it is well-known that the volatile fraction of a plant can also reliably be represented by its headspace composition, that is, a sampling approach that can be combined on-line to the subsequent GC or GC-MS analysis, thus making it possible to develop a one-step fully automated and time-saving method to discriminate between the two chemotypes. The headspaces of different parts of the plant for both chemotypes were sampled through a high-concentration-capacity HS sampling technique, to obtain significant GC profiles. SPME, first introduced by Pawliszyn (*25*) in 1993, is well established in the field of aromatic and medicinal plants (*26*, *27*): in HS-SPME the analytes from the vapor or gaseous phase, in equilibrium with a solid or liquid sample, are directly absorbed and/or sorbed onto a polymer-coated fused-silica fiber and then recovered by thermal desorption into a GC injection port and directly analyzed by GC or GC-MS.

Several fibers are available commercially; a three-component fiber (CAR/PDMS/DVB) was found to be the most effective for this application. **Figure 5** reports the GC-MS profiles of the headspaces sampled by SPME of aerial parts, latex, and roots of both poisonous (**A1**, **A2**, and **A3**) and nonpoisonous (**B1**, **B2**, and **B3**) chemotypes. The HS-SPME-GC profiles of all parts of the plant investigated could easily be distinguished, thus affording unequivocal discrimination between the two chemotypes. **Table 4** also reports the percent areas of the components identified in the headspaces sampled by SPME of aerial parts, roots, and latex of both *F. communis* chemotypes. It should be noted that analysis of the volatile fraction through its headspace also provided reliable profiles of the latex volatile fractions, unlike when the essential oil was sampled, probably because of the limited amount of latex available. In this case, also, the poisonous chemotype was characterized by aristolene, which was present at high percentages in all parts of the plant, together with β-gurjunene. On the other hand, allohedycaryol characterized the nonpoisonous chemotype, being the main component in the headspaces of roots and latex, although to different extents, and predominating in leaves, where the most abundant components were *trans*-β-ocimene and myrcene. In the roots and latex headspace, jaeschkeanadiol and traces of another daucane derivative, with a retention index (RI) of 1700 on an OV1 column (**Table 3**), were found, giving further confirmation of the identity of the nonpoisonous chemotype; moreover, significant percentages of an unidentified sesquiterpene alcohol (indicated in the essential oil as RI 1693 on the OV1 column) (**Table 3**) that was also detected in the essential oil were found. Small amounts of aristolene were also detected in the headspace of the roots of the nonpoisonous chemotype, whereas traces of allohedycaryol were found in the roots and latex of the poisonous chemotype.

In conclusion, this study has shown that chemical analysis is an effective tool to discriminate poisonous from nonpoisonous chemotypes of giant fennel that cannot be distinguished botanically. Their discrimination has here been shown to be possible not only by analyzing their biologically active fractions by HPLC-DAD-UV-MS but also through that of the volatile fraction (essential oil-GC-MS or HS-SPME-GC-MS). HS-SPME-GC-MS has also been shown to be successful as a one-step fully automated method to discriminate unequivocally between the two chemotypes; aristolene and allohedycaryol can be used as markers to distinguish the poisonous from the nonpoisonous chemotype.

Supporting Information Available: Linear regression equations, correlation coefficients, LOD, and LOQ for each investigated standard component. This material is available free of charge via the Internet at http://pubs.acs.org.

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