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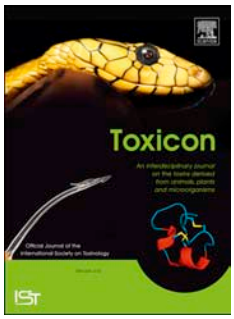

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Variability of ferulenol and ferprenin concentration in French giant fennel *(Ferula sp.*) leaves

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ABSTRACT

Few studies have reported quantitative data about the levels of prenylated coumarins in *Ferula* sp. Yet, the toxicity of *Ferula sp.* is only due to the presence of prenylated coumarins and to their concentrations and all studies suggest the existence of several chemotypes within the same species or even within the same variety.

The aim of this study was to investigate the hypothesis of different chemotypes in french *Ferula sp.* in relationship with the botanical species. In this objective, the species of giant fennels and their concentrations in prenylated coumarins were explored.

Three different species or subspecies of giant fennel were detected in continental France: *F. communis communis communis* L., *F. communis catalaunica microcarpa* Cauwet-Marc and *F. glauca* L. Surprisingly, the three species/subspecies of giant fennels were found to be located in exclusive and well separated geographical areas. In French giant fennels, ferulenol and ferprenin were detected. Distribution of ferulenol and ferprenin were found to be different between botanical varieties, but also according to the season, the soil and the altitude.

Our study seems to suggest that among *F. communis* species, the same plant can be regarded as poisonous at one point and another as non-poisonous.

Keywords : Ferula communis, Ferula glauca, Distribution, Prenylated coumarins, Ferulenol concentration, Ferprenin concentration,

Abbreviations : VKORC1, Vitamin K epoxide reductase complex subunit 1; DM, dry matter

1. INTRODUCTION

The genus *Ferula* belongs to the family Apiaceae. This genus contains more than 130 species found from central Asia throughout the mediterranean area to northern Africa (Cauwet-Marc, 1981; Infante, 1965; Kurzyna-Młynik et al. 2008; Pimenov and Leonov 2004). They are all robust plants, up to several meters in height, with yellow flowers in bloom in May-July according to the species. In France, presence of three species was observed; *F. arrigonii* Bocchieri, *F. communis* L. and *F. glauca* L (Reduron, 2007). (Table 1).

Even if *F. glauca* was initially considered as subspecies of *F. communis*, it is now considered as a different species because of its morphological, anatomical and ecological differences with *F. communis*. *F. glauca* differs from *F. communis* in particular by the morphology of its leaves. In France, *F. glauca* was described to be present only in the Languedoc region (Reduron 2007). This species was reported to be absent in Corsica. Moreover, in France, distribution areas of this species were reported to be different from distribution areas of *F. communis*. *F. glauca* was considered to be not toxic because prenylated coumarin was not detected (Lamnaouer, 1987; Maggi et al. 2009). Nevertheless, only analysis of essential oil of *F. glauca* collected in Pioraco in the center of Italia are available. Extensive analyses are necessary to complete these first phytochemical descriptions.

F. communis is the most widespread species of the genus in France. Among this species, several subspecies and varieties exist, but their characterization and their taxonomy are still not clear. In France, three varieties – i.e., *communis*, *brevifolia* and *microcarpa Cauwet-Marc*, were described (Table 1). This species was shown to be responsible for an hemorrhagic syndrome in livestock (Benkhalti and Lamnaouer, 1994; Cauwet-Marc, 1990). This syndrome was called ferulosis. This syndrome is due to the presence of prenylated coumarins (Appendino et al. 2001; Appendino et al. 1988a; Fraigui et al. 2001; Miski and Mabry, 1985; Rubiolo et al. 2006; Valle et al. 1986), – i.e., ferulenol and ferprenin (Appendino et al. 1988b; Carboni et al. 1964) in all the parts of the plant (leaf, root, flower, stem). Others ω-oxygenated prenylated coumarins were also described to be present but they must play a secondary role in the syndrome due to rapid elimination by the organism of the animal due to the presence of oxygenation (Appendino et al. 1988a). These prenylated coumarins are specific inhibitors of the VKORC1 enzyme of all mammals, even if ferulenol was shown recently to be a better inhibitor of VKORC1 than warfarin (Gebauer, 2007) and ferprenin (Louvet et al. 2015). The VKORC1 enzyme is expressed in the liver of all mammals. This enzyme is responsible for the recycling of vitamin K essential for the activation of clotting factors II, VII, IX and X (Oldenburg et al. 2008). After consumption of *F. communis* containing ferulenol and ferprenin by livestock, the blood clotting is impaired leading to ferulosis (Shlosberg and Egyed 1985). This intoxication affected almost all mammals. Even man may be affected after an uncontrolled therapeutic use of Ferula extract (Cornevin, 1887; Lannehoa et al. 1998). In spite of the wide geographical distribution of *F communis***,** ferulosis appears to be limited to a few areas of the western Mediterranean region, especially Algeria, Marocco, Tunisia, Sardinia, Italia, Spain and Israel in the eastern part (Infante, 1965; Reduron, 2007; Shlosberg, 2011). This would be due to the presence of different chemotypes of *F. communis*: the 'non-poisonous' chemotype and the 'poisonous' chemotype associated to

the hemorrhagic syndrome (Benkhalti and Lamnaouer 1994; Sacchetti et al. 2003). Both chemotypes have been reported to show no morphological differences and to have the same somatic chromosome number (2n=24). On the other hand, in 'non-poisonous' chemotypes, the main constituents would be daucane esters while in 'poisonous' chemotypes, the main constituents would be the prenylated coumarins, even if presence of ferulenol was also described in 'non-poisonous' chemotypes (Arnoldi et al. 2004; Rubiolo et al. 2006). The causes of the difference in contents between chemotypes are absolutely not known. In France, *F. communis* was described all along the Mediterranean coast and in Corsica, but the only intoxications of animals were reported in Corsica. The National Centre of Veterinary Toxicology of Lyon (France) diagnosed only 28 clinical cases of giant fennel intoxication in cattle, sheep, pig and horse from 1990 to 2013 in France (Gault et al. 2015). No information about the chemotype of the French *F. communis* is currently available.

Until now, few studies have reported quantitative data about the levels of prenylated coumarins in *Ferula* sp. Yet, the toxicity of *Ferula sp.* is only due to the presence of prenylated coumarins and to their concentrations. Moreover, for the few studies reporting quantitative results (Arnoldi et al. 2004; Rubiolo et al. 2006; Sacchetti et al. 2003), differences in the analytical methods make interpretation of results difficult. Nevertheless, all studies suggest the existence of several chemotypes within the same species or even within the same variety. The aim of this study was to investigate the hypothesis of different chemotypes in french *Ferula sp.* in relationship with the botanical species and to produce original data about their ferulenol and ferprenin contents.

2. MATERIALS AND METHODS

2.1. Chemical syntheses

Ferulenol was synthesized accordingly to Gebauer et al (Gebauer, 2007). Oxidation of trans,trans-farnesol with PDC afforded farnesal in 87% yield (Hu et al. 2004). Ferprenin was obtained by reaction of 4-hydroxycoumarin with 2 equivalents of trans,trans-farnesal in water at 80°C, in a Knoevenagel / electrocyclization tandem mechanism (Jin Jung et al. 2010)(SI 1). The purities of ferulenol and ferprenin were checked by NMR and were higher than 98%.

2.2. Plant material

Basal young leaves of 199 samples of *Ferula* sp. were collected in France (Figure 1). 172 samples were picked from different spots along the Mediterranean coast of France, from the Spanish border in the south to the limit of the French Riviera in the east. The Mediterranean coast is divided into two geographical parts by the Rhône River flowing from north to south. Samples were harvested in the north part, other in the South part. Samples were also taken from the back country of Nîmes and along the Rhône River as far as 150 km from the coast. 27 samples were picked in Corsica (20 from North Corsica and 7 from South Corsica). No sample was picked from the far south of Corsica (Bonifacio area) where *Ferula arrigonii* was growing. The sampling spots were chosen in accordance to the information of Cauwet-Marc (Cauwet Marc 1988). Moreover 19 dry samples from different Mediterranean countries were analysed: 7 samples from Algeria, 3 from Tunisia, 3 from Italia, 2 from Spain and 4 from Portugal. All french samples (continental and corsican) were picked in january and february at the early beginning of the growth. Additional samples of *Ferula communis* were picked in june of the same year. The others were sent us as dry leaves. *Ferula sp* were freely widespread in the investigated areas at least when they were preserved from agricultural practices. The free access to the plant is depending on the farming system; free grazing is very common in Corsica (cattle, sheep, goat and wild boar) and in the far south of continental

France (sheep mainly, wild boar) as in North Africa (Morocco, Algeria, Tunisia). Clinical and subclinical toxicity is surely underevaluated.

The botanical determination was performed by the authors (Gilbert Gault (DVM, botanist) and Denis Grancher (DVM, PhD, Assoc. Pr in Poisonous plants)) in accordance with the data provided by Cauwet-Marc (Cauwet Marc 1988; Cauwet-Marc 1981) and Reduron (Reduron 2007). It was based on morphological considerations: size of the plants (*F. communis* being taller and stronger than *F. microcarpa* and *F. glauca*), colour of the leaves (which was full green on each side in both *F. communis* when it was sea green in *F. glauca*), size of the fruits (smaller in *F. microcarpa*) and date of blooming (much earlier in *F. communis* than in *F. microcarpa* and *F. glauca* in same weather conditions). One or two leaves were sampled from one plant at each spot and put into opaque paper bags. No voucher sample was deposited but all dried and powdered samples are still stored lightproof at room temperature.

2.3. Plant treatment

In two days after picking all samples were oven dried at 60°C during 24 hours (Jouan-Paris). After drying the dry matter was ground (Tecator-Sweden, particle size : 500 µm) and stored in polypropylene tubes away from natural light. 70 of the 199 samples were precisely dried to measure the dry matter content of the leaves. The foreign samples were dried at room temperature of the respective countries.

2.4. Extraction of ferulenol and ferprenin

Ferulenol and ferprenin were first extracted from the dry samples of giant fennel (Rubiolo et al. 2006). Each dry sample (100 mg of powder) was put into a 50 ml polypropylene tube, added with 4 ml acetonitrile and stirred 30 seconds at 20000 rpm. The supernatant was filtered and poured in a new tube. The dry sample was extracted again with 4 ml acetonitrile,

149 stirred, filtered and separated. A third extraction took place with 2 ml acetonitrile. The three
150 supernatants were gathered then added with 8 ml hexane and vigorously shaken. The heavy
151 fraction (acetonitrile extract) was separated, stored in a borosilicate tube and evaporated under
152 air flow at 37°C. The dry residue was diluted with 1 ml methanol and stored before
153 measurement of ferulenol and ferprenin content. The stirring apparatus was cautiously and
154 meticulously cleaned and brushed with acetonitrile and methanol between each extraction.
155 Blanks were prepared according to the same procedure of extraction as the samples.

156

157 **2.5. Ferulenol and ferprenin analysis by LC-MS**

158 Chromatographic analysis was performed on an Agilent 1100 series HPLC (Agilent
159 Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved on XBridge
160 C18 column (4.6 * 150 mm, 5 µm, Waters, Milford, MA, USA) at 48°C with a mobile phase
161 of methanol, 0.1% formic acid (96:4) in isocratic condition. The flow rate was 0.5 mL/min.
162 The injection volume was 20 µL, and the autosampler tray was at ambient temperature.
163 MS detection was carried out by a 6120 Quadripole (Agilent Technologies, Santa Clara, CA,
164 USA) equipped with an Atmospheric Pressure Chemical Ionisation (APCI) interface and a
165 LCMS Chemstation software from Agilent Technologies (Palo Alto, CA, USA). Detection
166 was by MS with APCI source in positive mode. Nebulizer pressure was set to 60 psi, dry gas
167 temperature to 350°C, dry gas flow to 5 L/min, and vaporizer temperature to 400°C. Capillary
168 voltage was set to 4000V, corona needle to 10µA. Identification criteria were the retention
169 time (tr= 7.0-7.3 min for ferulenol and 8.4-8.8 min for ferprenin) and the selected ion (367 for
170 ferulenol and 365 for ferprenin after positive ionization $M_{ion} = M_{molar} + 1$). (figure 2)
171 Concentrations of ferulenol and ferprenin were calculated in reference to recurrent injections
172 of standard solutions and blanks every 5 or 6 samples.

2.6. Statistical analysis

Statistical analysis were performed with R v. 3.1.2. Owing to the distribution of our data, which did not follow a normal distribution, non-parametrical tests were performed. To compare the different groups, a Kruskal-Wallis test was performed. If it was significant, we used a pot hoc Dunn's test to compare between groups.

3. RESULTS

3.1. Three varieties of *Ferula* were found in France

199 leaves samples of *Ferula sp.* were collected in France, 172 from continental France and 27 from Corsica. Leaves were identified to belong to different species or subspecies of *Ferula sp.* according to the morphological criteria described in the materials and methods (Table 1). Concerning the samples collected in continental France, 140 leaves were collected from *F. communis communis* (noted *F. communis* in the following), 12 from *F. communis catalaunica microcarpa* (noted *F. microcarpa* in the following) and 20 from *F. glauca*. Concerning the samples collected in Corsica, all the leaves were collected from *F. communis communis*.

In continental France, the geographical repartition of the three varieties of Ferula was clearly different. *F. communis* was sampled only between Marseille and Toulon, in the east of the Rhône River. *F. microcarpa* was found only in the far south of the French Mediterranean coast, close to the Spanish border and *F. glauca* was identified and picked only in the west side of the Rhône river (Figure 1). These areas were exclusive except to the north of our harvest area (in the Rhône valley, 150 km far from the coast) where large scale public works were carried out (highways, high speed railways for example) with mixtures of soil from different origins along the Rhône valley.

The dry matter of the fresh leaves of giant fennel were respectively 18.39 % (SD= 3.28%, N=38) for *F. communis*, 22.76% (SD=2.61%, N=12) for *F. microcarpa* and 23.74% (SD=1.87%, N=20) for *F. glauca*. The dry matter content of *F. communis* was significantly lower than the dry matter of *F.microcarpa* ($p<0.001$) and *F.glauca* ($p<0.001$). *F.microcarpa* and *F.glauca* were not significantly different ($p=0.125$).

3.2. Ferprenin and ferulenol were detected in *Ferula sp.*

In extracts of leaves of *F. communis*, ferulenol presented a ionized molecular mass of 367 with a retention time of 7.0-7.3 min corresponding to the retention time of the synthesized ferulenol. Results of concentrations of ferulenol determined in leaves of continental French *Ferula sp.* are presented in Figure 3. Ferulenol was detected in *F. communis* (average = 666.2 µg/g DM, N=141) and *F. microcarpa* (average = 95.1 µg/g DM, N=14), but was almost undetectable in *F. glauca* (average= 0.6 µg/g DM, N=21). Concentration of ferulenol was all significantly different between varieties of *Ferula*.

Ferprenin presented a molecular mass of 365 with a retention time of 8.6-8.8 min corresponding to the retention time of the synthesized ferprenin. It was detected in both *F. communis* (average = 222.3 µg/g DM, N=140) and *F. microcarpa* (average = 70.1 µg/g DM, N=12) but was absent in *F. glauca*. Its concentration was significantly different between *F. communis* and *F. microcarpa*.

3.3. Comparative analysis of ferulenol and ferprenin amounts according to the plant and the geographical area

In continental varieties of giant fennel the levels of ferulenol and ferprenin were widely spread. Figure 3 presents the concentrations of ferulenol and ferprenin per plant. Ferulenol ranged from 1.19 to 8331 µg/g DM in *F. communis* ; from 13.7 to 849 µg/g DM in *F. microcarpa* ; and from 0.0 to 4.8 µg/g DM in *F.glauca*. The levels of ferprenin ranged from 0.0 to 1497.4 µg/g DM in *F.communis*; from 0.0 to 274.3 µg/g DM in *F.microcarpa*.

In corsican giant fennel (i.e. *Ferula c.c. communis*) the levels of ferulenol and ferprenin were also widely spread. Table 2 and Figure 4 show the global and detailed results of the Corsican samples comparatively to samples of metropolitan France. In *F. communis* of South Corsica (N=7), ferulenol ranged from 15.6 to 1495.9 µg/g with a median of 61.5 µg/g DM) and ferprenin ranged from 0 to 31.4 µg/g DM. In *F. communis* of North Corsica (N=20), ferulenol ranged from 0 to 86.7 µg/g with a median of 29.4 µg/g DM, and ferprenin ranged from 0 to 414.7 µg/g DM with a median of 49.8 µg/g DM.

Concentrations of ferulenol and ferprenin determined in leaves of French *F. communis* were compared to concentrations of leaves of *F. communis* received from various Mediterranean countries. Analyses confirmed the very broad variation of the levels of ferulenol and ferprenin between geographical areas (table 2). Ferulenol was detected in leaves of *F. communis* picked up in all Mediterranean countries. However concentration of ferulenol was almost 50-fold higher in *F. communis* of Italy and Algeria compared to those of continental France and 10-fold lower in *F. communis* of Portugal and Tunisia.

3.4. Impact of season, soil and altitude on ferulenol and ferprenin amounts in *F. communis*

To perform this analysis, only *F. communis* leaf samples in South East of France close to Marseille and Toulon were considered.

Concentrations of ferulenol and ferprenin were evaluated according to the sampling period. Eleven samplings were done in February, 10 in April, 9 in June and 2 in November. Results are shown in Figure 5. For ferprenin concentrations, no influence of the season was observed. On the contrary, an influence of the sampling period was noticed for ferulenol. Ferulenol concentrations in *F. communis* were significantly higher in June than in February and April. Concentrations of ferulenol and ferprenin were also analyzed according to the altitude of the sampling and the type of soil. Because of the influence of the season, only samples of *F. communis* done in June (i.e., corresponding to month with the highest concentration) in the South East of France were used for ferulenol analysis, while only samples of *F. communis* done in February in the South East of France were used for ferprenin analysis. Results are shown in Figure 6. Altitudes of *F. communis* sampling points were comprised between 0 and 300 meters. Concentrations of ferulenol in the leaves of *F. communis* were not significantly different according to the altitude of the sampling points. They were also not significantly different according to the soil type. On the contrary, concentrations of ferprenin significantly decreased when altitude decreased at least between 0 and 165 meters. Moreover, ferprenin concentration was significantly influenced by the soil type. *F. communis* growing in sand presented the highest concentrations of ferprenin with concentrations 4-fold higher compared to *F. communis* growing in limestone or schist.

4. DISCUSSION-CONCLUSION

In this study, three different species or subspecies of giant fennel were detected in continental France: *F. communis communis communis* L., *F. communis catalaunica microcarpa* Cauwet-Marc and *F. glauca* L. as described previously in the 1980'. In France, these giant fennels were observed only in the south below a latitude of 45 ° north. Curiously, even if all the giant fennels are found in the South of France, the three species/subspecies of giant fennels are located in exclusive and well separated geographical areas. *F. communis* is present

exclusively in the south East of France and Corsica, *F. microcarpa* in the South West of the Mediterranean coast of France and between the two areas, in the center of the South of France, are present the species *F. glauca*. An influence of the topsoil or the subsoil on that particular distribution may be considered. *F. glauca* were all harvested on jurassic or cretaceous limestone. Except for two *F. microcarpa* harvested on a metamorphic subsoil (chlorite schists), the other *F. microcarpa* were also harvested on jurassic or cretaceous limestone subsoil. Some *F. communis* were also found in areas with limestone subsoil, even if subsoils of geographical areas where *F. communis* are observed are more heterogeneous with subsoils of schist, sand or limestone. Anyway, since the three species can grow on limestone, influence of the nature of the subsoil on this surprising distribution seems to be ruled out. Other factors could possibly be considered, topography, average annual sunshine, hydrological features, prevailing winds…. But the results obtained in this study do not allow to explore these hypotheses.

After exploring the species of giant fennels present in France, their toxicity has secondly been explored. Toxicity of Ferula was reported to be closely related to its concentration in prenylated coumarins. Indeed, among *F. communis*, two different chemotypes were reported, the “non-poisonous” chemotype containing essentially daucane esters and the “poisonous” chemotype containing essentially prenylated coumarins, the ferulenol, a 4-hydroxycoumarin derivative and the ferprenin, a pyrane (3,2-c) coumarin derivative (Appendino et al. 2001; Appendino et al. 1988a; Fraigui et al. 2001; Miski and Mabry, 1985; Rubiolo et al. 2006; Valle et al. 1986). A recent study demonstrated that ferulenol and ferprenin are both effective to inhibit the VKORC1 enzyme (Louvet et al. 2015), thereby preventing the recycling of vitamin K in animals that consumed enough *Ferula sp.* containing one of these molecules. Ability of these molecules to inhibit VKORC1 enzyme are very similar between species, which means that all animals consuming Ferula containing one of these molecules in

sufficient quantity could present bleeding disorders. Presence of other ω-oxygenated prenylated coumarins have been reported. Nevertheless, the ω-oxygentation is certainly responsible for a very rapid elimination of these compounds by phase II drug metabolizing enzymes. That is why we systematically determined in this study the concentration of ferulenol and ferprenin in leaves of French Ferula to better manage the risks associated with the presence of these plants. Concentrations were determined in the leaves because it is the part of the plant that is generally easily devoured by livestock and therefore is mostly involved in poisoning. Results were expressed as µg of active substance per gram of dry matter.The significant differences between levels of dry matter in the leaves of *F. Communis* vs *F. microcarpa* and *F. glauca* confirms the interest of this measurement in the expression of the results of analysis. Moreover drying the samples allows to preserve them a longer time.

Ferprenin was only detected in *F. communis* and *F. microcarpa* with concentrations lower than those of ferulenol. Although ferprenin is also able to inhibit VKORC1, its inhibiting power is significantly lower than that of ferulenol (Louvet et al. 2015). Thus, the toxicity of French *F. communis* is certainly more associated to the quantities of ferulenol they contain than those of ferprenin unless the quantities of ferulenol and ferprenin differ in the other parts of the plant.

Among the French *ferula*, the species and subspecies that seems to be the most dangerous is *F. communis* growing in the east side of the French Mediterranean coast and in Corsica. Indeed, concentrations of prenylated coumarins found in the leaves of *F. communis* are much higher than those measured in other species/subspecies. This is totally coherent with clinical cases of ‘ferulosis’ observed in France. These clinical cases were only reported in Corsica where only *F. communis* is growing (except one spot of *F. arrigonii* Bocchieri in the far south of the island). Nevertheless, because concentrations of ferulenol and ferprenin are much higher in continental *F. communis* compared to Corsican *F. communis*, the continental giant

fennel should be more dangerous than the corsican one. The very rarely suspected clinical cases in the continental area of growth of *F. communis* may be related to the very small number of domestic animals in this area and/or an extensive but monitored farming system. On the contrary almost all Corsican domestic animals (horse, cattle, pig) are systematically free ranged with total freedom in spring, summer and autumn.

Surprisingly, concentrations of prenylated coumarins in the leaves of *F. communis* are highly variable between plants with concentrations varying from 1 to 8000 µg/g DM for ferulenol, and from 0 to 1500 µg/g DM for ferprenin in France. This broad variability in concentrations of prenylated coumarins observed in France is also observed between Mediterranean countries. Our data give evidence of much higher levels of ferulenol in Italian and Algerian *F. communis* compared to France, while in *F. communis* harvested in Portugal and Tunisia, concentrations of ferulenol are very low. These results are consistent with the earlier description of two chemotypes of *F. communis* in Sardinia, the 'poisonous' chemotype and the 'non-poisonous' chemotype. Nevertheless, our study seems to suggest that the same plant can be regarded as poisonous at one point and another, as non-poisonous. Indeed, some factors appear to influence the presence of prenylated coumarins. Indeed, production of prenylated coumarins by *F. communis* could vary according to the season. Our results suggest that production of ferulenol by the plant may be stronger in June. Indeed, the leaves collected from January to April contained almost no ferulenol, while those collected in June exhibited very high concentrations of ferulenol. The concentrations of ferprenin seem rather decreased from January to June with concentrations in the leaves higher in February than in June. This influence of the season observed in this study may be related to various factors, the vegetative stage of the plant, precipitation, sunshine, temperature…. Other factors could also influence the amounts of coumarin derivatives present in the leaves of *F. communis*. A negative correlation was found between altitude and concentration of ferprenin. The soil may also have

an influence. Indeed, concentrations of ferprenin are much stronger in the leaves of *F. communis* growing in the sands than those growing on limestone or schist. Therefore additional studies seem necessary to identify the influence of these various parameters on the concentrations of prenylated coumarins. Moreover, the presence or absence of ferulenol and/or ferprenin residues in liver of domestic or wild animals suffering from ferulosis should be studied in order to analyze the role of each prenylated coumarin in the syndrome.

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LEGENDS OF FIGURES

Figure 1: Distribution of the different species of giant fennel in the south of France

Figure 2 (A-D): Chromatograms of ferulenol and ferprenin (LC-MS). A: example of ferulenol standard (M+1=367, rt=7.045 min). B: example of ferprenin standard (M+1=365, rt=8,488), C: sample 0012 for ferulenol, D: sample 0012 for ferprenin.

Figure 3: Influence of Giant fennel species on the level of Ferulenol and Ferprenin in leaves.

Figure 4: Influence of geography location on the level of ferprenin (A) and ferulenol (B) in leaves of Giant Fennel.

Figure 5: Influence of season on the level of ferprenin and ferulenol in leaves of Giant Fennel. To avoid interaction, only samples harvested near Toulon are assessed.

Figure 6: Influence of altitude and the soil on the level of ferprenin and ferulenol in leaves of Giant Fennel. To avoid interaction, only samples which have been harvested near Toulon and in June for ferulenol, or in February for ferprenin are assessed.

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Genus	Species	Subspecies	Varieties
Ferula	***communis* L.**	***communis***	***communis***
		brevifolia	*brevifolia*
		genuina BURNAT	
		catalaunica	*catalaunica*
			***microcarpa* CAUWET-MARC**
	***glauca* L.**		
	tingitana L.		
	***arrigonii* BOCCHIERI**		

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482 **Table 1** : Species, subspecies and varieties of *Ferula sp*. The present varieties in France

483 including Corsica are in bold letters in colored cells.

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***F. communis communis* from**	**N**	**Ferulenol** (µg/g DM)	**Ferprenin** (µg/g DM)
Continental France	140	149.7 (37.9-562.4)^{A}	181.8 (112.4-271.4)^{ABC}
Corsica			
from South	7	61.5 (34.9-853.3)	0 (0-16.2)^{A}
from North	20	29.4 (12.9-44.5)^{ABC}	49.8(25.1-100.1)^{B}
Algeria	7	4853 (2461-7133)^{BDE}	75.7 (0.0-174.2)
Italia	3	5765 (3198-6488)^{CFG}	0.0 (0.0-402.4)
Portugal	3	6.5 (1.5-9.3)^{DF}	0.0 (0-0)^{C}
Spain	1	138.8	0.0
Tunisia	3	29.8 (4.5-32.1)^{EG}	52.6 (3.9-76.9)
Kruskal-Wallis P-value		<0.0001	<0.0001

Table 2 : Levels of ferulenol and ferprenin in Giant fennel leaves of France and other Mediterranean countries. Values are presented as median and numbers in brackets are the first and the third quartile. N is the number of samples. Values in the same column with a same superscript letter are significantly different.

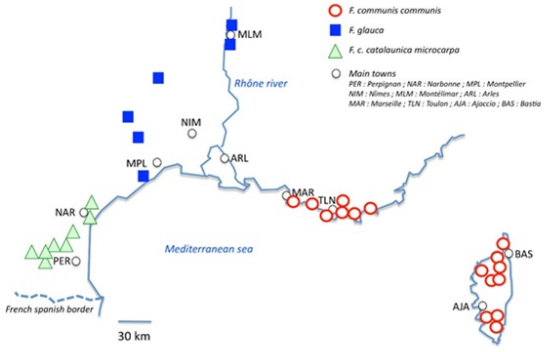


Figure 1

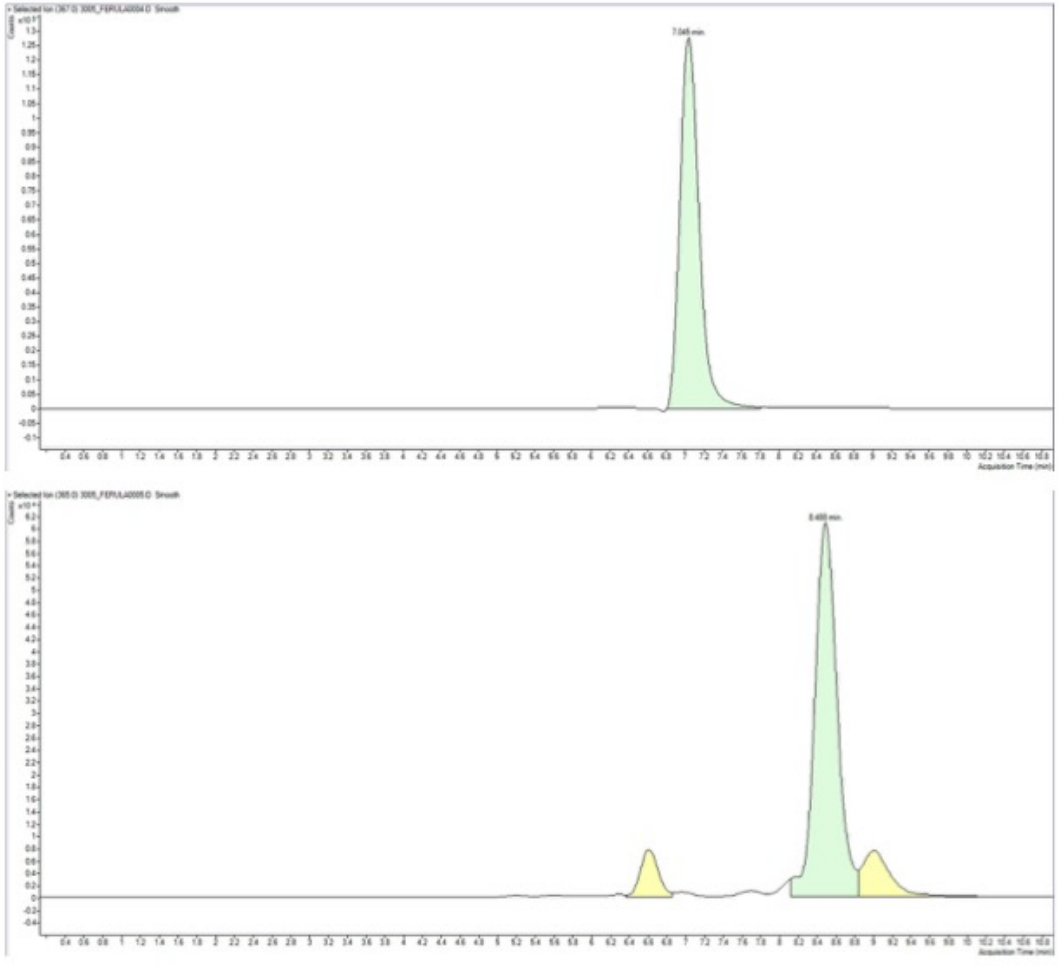


Figure 2, A & B

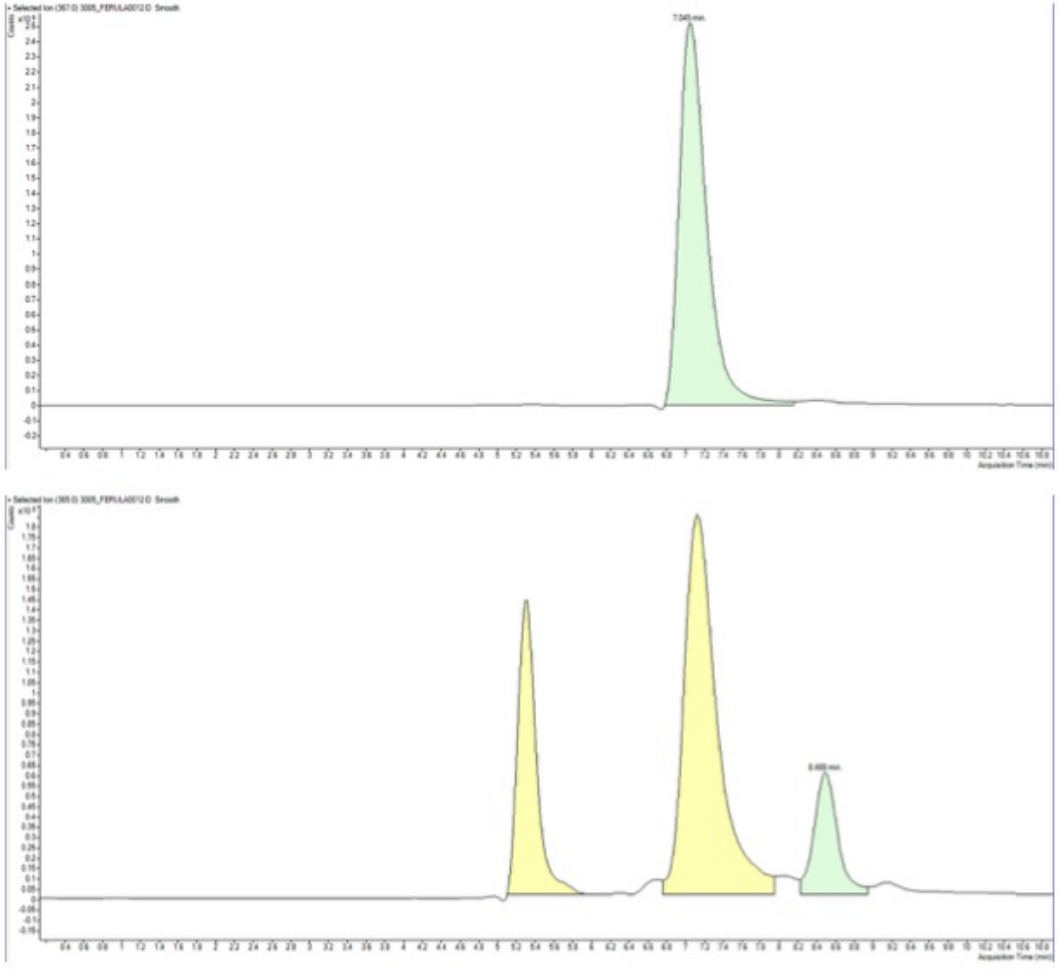
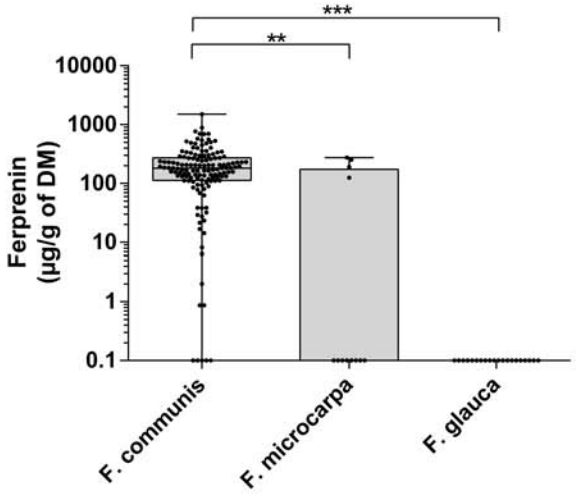
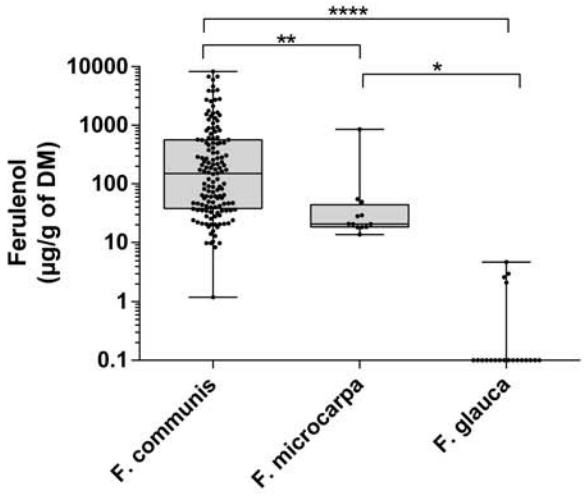
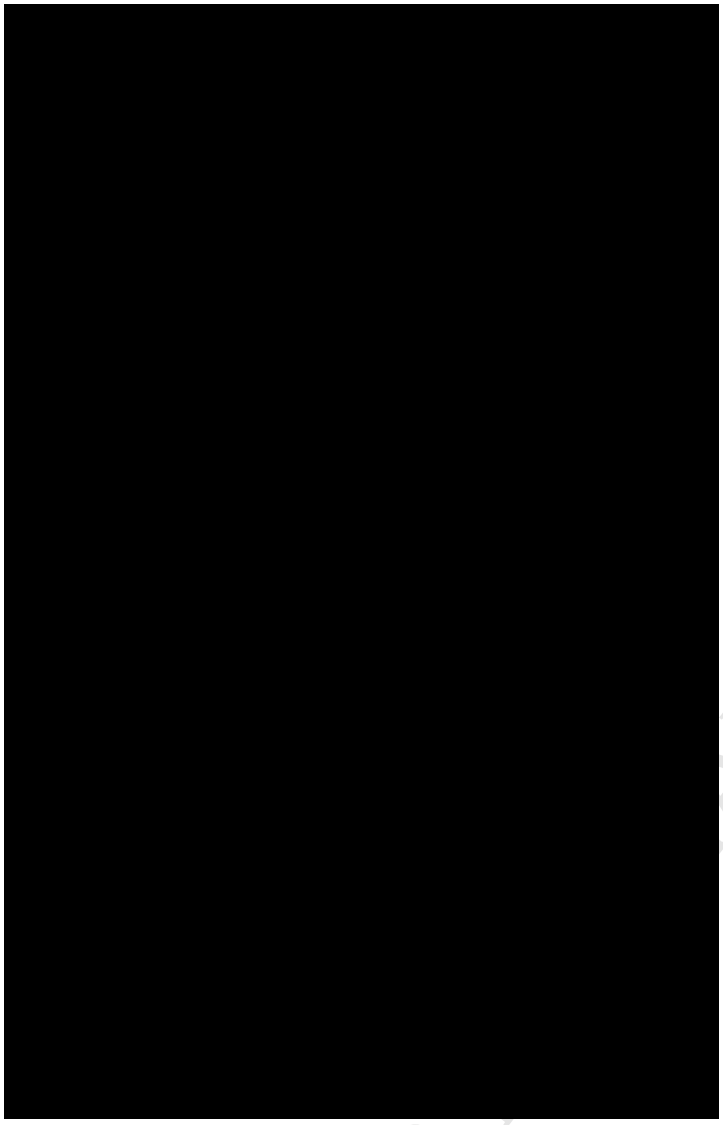
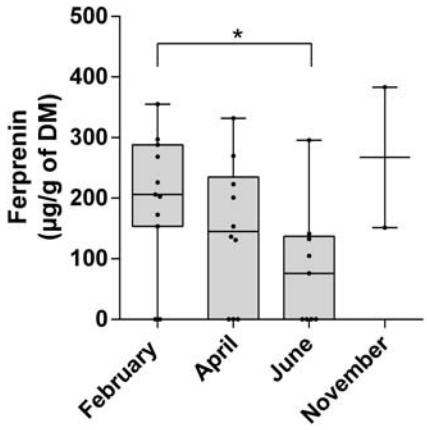


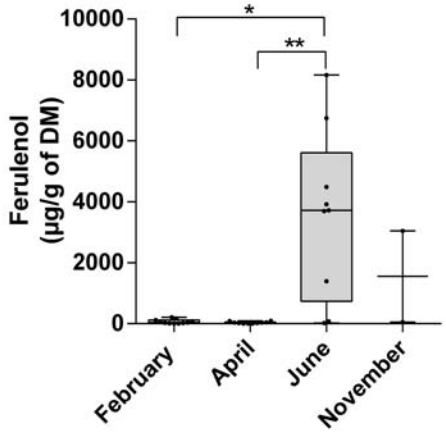
Figure 2, C & D



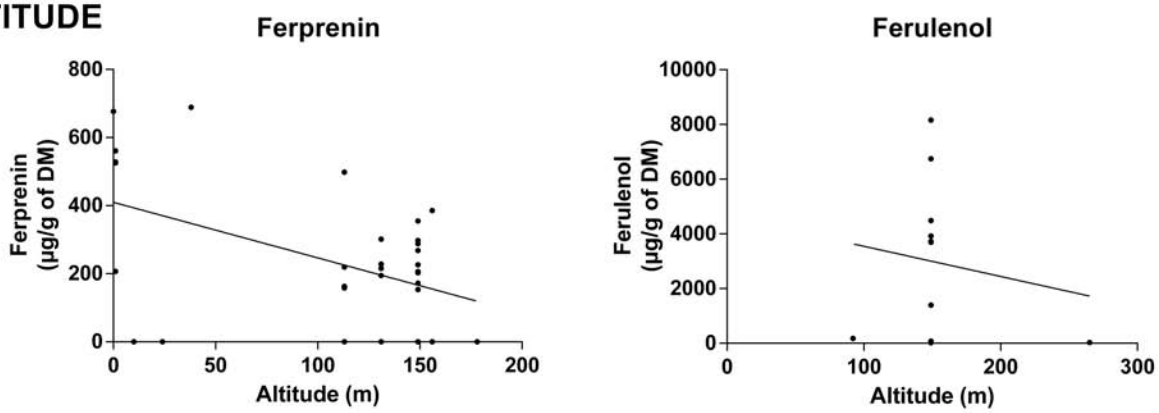




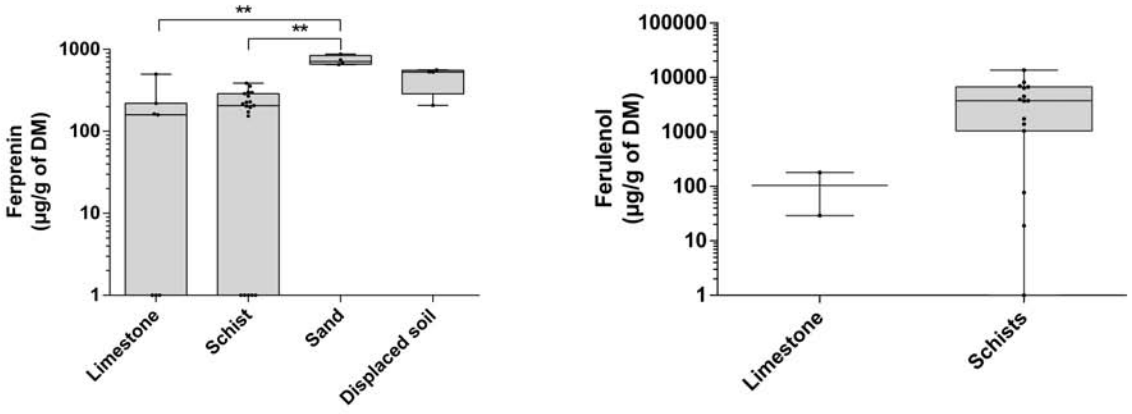




ALTITUDE



SOIL



1. *Ferula communis* is the most widespread species of the genus in France
2. Ferulenol and ferprenin were detected in leaves of *Ferula* of France
3. Distribution of ferulenol and ferprenin is different between botanical varieties
4. Concentrations of prenylated coumarins are highly variable between plants
5. Season, soil and altitude seem to influence concentrations of prenylated coumarins