FERULA GUMMOSA: PHYTOCHEMICAL VARIABILITY IN IRAN

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Introduction

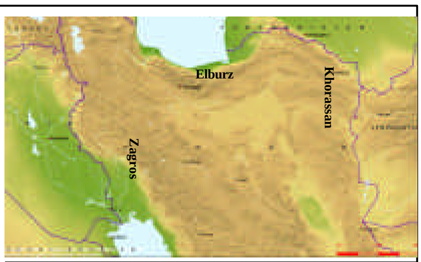
More than 20 species of the genus Ferula (Apiaceae) grow in Iran. Most of them produce resins with distinct phytochemical properties. The area of repartition extends through the mountainous and savannah-like regions with sub-desert climate.

The indigenous name "Galbanum" explicitly refers to the species Ferula gummosa. During the last decades, numerous adulterations of galbanum with resins from other Ferula species, and sometimes completely unrelated species, were sold internationally, especially on the European market. As there is no traceability of the raw material, its quality and purity is constantly decreasing.

Our study was aimed on taking samples of resins of strictly identified plants during their growth period between mid May and mid June, and to analyse their phytochemical composition. The analyses were performed by the Service Central d'Analyse du CRNS (Centre National de Recherche Scientifique) in F-69390 Vernaison (France).

For our field study, we chose typical habitats of Ferula gummosa in geographical areas with distinct differences in geology, geography and climatic conditions:

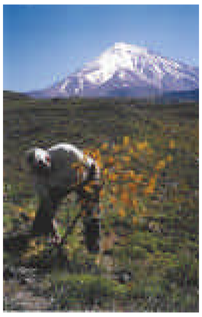
- Khorassan in the North-Eastern parts of Iran, close to the borders to Afghanistan and Turkmenistan.
- The mountain range of central Elburz.
- The montains of Zagros in the region of Isfahan and Kashan.



Vegetative cycle of *Ferula gummosa*

In contrast to literature data the only natural way of reproduction of Ferula gummosa is by seed. A vegetative multiplication starting from the rhizomes does not take place. This observation was confirmed to us by local botanists studying the botanical characteristics of the genus.

The seed germinate in the growing season following seed formation. Depending on the climatic conditions and the thaw of snow, two small leaves and a rootlet appear in March/April. From May/June on, the plantlet is dry and will only restart its vegetative cycle in springtime of the following year. Then, one or two additional leaves are formed, and some weeks later the plant disappears again.



On the three year old plants, an excressence forms in the upper parts of the root, developing into a rhizome in the course of the following growing seasons. It is this rhizome that later on yields the resin. After 7 or 8 vegetation cycles, an inflorescence is formed, flowering in May and bearing fruit in June. In central Elburz, the flowering takes place later. After the ripening of the fruit, the plant dies.

Fig.: Flowering *Ferula gummosa* in the Elburz mountain range

Impact of climate on populations

Ferula gummosa grows in altitudes between 1400 and 3200 m. Usually, it is not found in altitudes higher than 2.700 m in Khorassan, and 2.900 m in the Zagros mountains.

The populations are closely linked to the winterly rain and snow falls, especially in lower altitudes. In the past years, the decrease of rainfall had an impact on the density of Ferula populations. In certain habitats in low altitudes of especially dry mountain ranges the plant has already disappeared. With increasing altitude, the plant is encountered more frequently. Still, the lack of snowfall in the past years is expressed in an absence of young plants. The breaking of the dormancy of the seeds depends on snowfall, and the germination to the water provided by the melting snow.

Due to a climatologic peculiarity, the situation is essentially different in central Elburz. Here, the Northern mountain slopes are inclined towards the Caspian sea, and thus do not only receive abundant rainfall, but also have fog crossing the mountain chain and redescending on the much dryer Southern slopes, oriented towards the desertic central areas of Iran. The Southern slopes are the natural habitat of Ferula gummosa, receiving additional water from the fog in the vegetation period. In addition, the germination ensured by abundant winterly snowfalls. In consequence, there are dense populations of Ferula gummosa in the high altitudes of the central Elburz mountains. One some slopes we counted populations of more than 5.000 plants per hectare.

Despite of the still satisfying population density in high altitudes, the populations grows thin in the lower regions of central Elburz. The Iranian government seeks to control the populations of galbanum by closely regulating the collection in certain regions.

Due to the weak rainfalls of the past years, the production of resin of the individual plants is distinctly lower than in more humid years. Within the areas included in our studies, the yield of resin was only 40% of the amount usually collected in years with normal rainfall. Possibly, there is also an impact on the phytochemical composition, subject to future studies.

Method of collection

Ferula gummosa preferably grows on steep hillsides. The resin is collected starting from the 4th vegetation cycle until the beginning of the flowering. Only plants with completely dry aerial parts with a full stop of vegetation are subject of harvesting. Plants with inflorescences are not used, with the exception of droplets of resins from the sprout caused by parasitic insects drilling holes (Curculionidae, Cerambicydae and Cicadaceae).

The earth is removed around the hypocotyl to gain access to the rhizome. The rhizome is traditionally incised on a length of 3-5 cm with a special iron cutting tool locally forged. Within a few minutes, droplets of a white resin start to form along the cut, solidifying within a few hours.

In our field study we found the fresh resin of the plants of central Elburz to be of yellow colour, whereas it was white in all other populations. In the region of Khorassan and Zagros, the resin from rhizomes exposed to sunlight tends to turn yellowish, and remains white when protected from the sun. The phytochemical changes are subject to future studies.

The resin is collected 4-7 days after the incision, with the help of a forged iron tool formed like a spoon. The already existing cut is re-incised, and the newly formed resin is collected one week later. The procedure is repeated a third time, rarely a fourth time. The yield of resin is growing with the number of incisions. In average, every plant gives approximately 20 g per season. One ton of galbanum is thus gained from 50.000 individual plants, and 150.000 incisions.

Analytical results

The analyses of the samples were performed by H. Casabianca from the Service Central d'Analyse du CNRS, F-69390 Vernaison (France).

Three major phytochemical groups can be differentiated from the analytical results, identified by relatively constant marker compounds. They largely correlate to the three geographic zones investigated in our study. A distinct phytochemical variability was not only found between the major investigated areas, but also between plants in the same habitats. Still, reproducible parameters typical for the geographic region could be identified.

As the collection of galbanum traditionally is a major source of income in the Khorassan region, our study focussed on the samples obtained from this area. In addition, samples of galbanum from the Iranian market and material originating from Afghanistan were also analyzed.

On storage, an essential oil separates from galbanum. This essential oil was separately investigated in samples from 100% pure galbanum. The essential oil is sometimes added to commercial lots of galbanum to "ameliorate" the quality, even though this measure will lead to a shift in phytochemical parameters. Within the oil, an inversion of the percentage of ?3-carene and ß-pinene relative to the resin is observed, together with an almost complete lack of undecatrienes (0.07%). Undecatrienes are a major constituent of galbanum. In our screening, we found plant populations where the contents of undecatrienes reached up to 4.2 percent.

An intriguing result of our analyses was the distinct difference between the composition of the resin from galbanum from strictly identified plants, and the specifications for galbanum laid down by the AFNOR. The differences found for the contents of a-pinene, ß-pinene, ?3-carene and undecatrienes point to a certain percentage of resin of foreign Ferula species in the official specification.

Adulterations

Within the past decades, the supply of galbanum was subject to economical and political changes in the countries of the Middle East. Actually, a major part of the supplies of galbanum seems to be more or less adulterated, or mixed with the resin of a Ferula species of Afghan origin. This resin is transported to the countries of the Persian Gulf via Pakistan, and re-exported from there on. Within our study, we were able to obtain pure resin of Afghan origin, and to identify its phytochemical components through GC analyses.

Recently, numerous samples of commercial galbanum supposedly originating from Iran were analyzed. The majority of the lots showed adulterations caused by middle men in the supply chain. Due to measures taken to ensure the traceability of the raw material and the cooperation of our Iranian partners, we could considerably augment the quality of commercial galbanum (object of a parallel study).

In our study of potential adulterants we also analyzed resins from other Apiaceae closely related to *Ferula gummosa*.

Ferula asafoetida Karst.

Ferula asafoetida Karst. (Apicaceae) spontaneously grows in many countries of the Middle East. In Iran, the plant exists in two varieties: one bitter, and one sweet. The use of the latter is preferred over the former. Even though *Ferula asafoetida* grows in more Southerly regions in Iran than Ferula gummosa, there are geographic areas where both species can be found. Its growth characteristics are similar to those of *Ferula gummosa*, but with a much longer vegetative cycle. The inflorescence forms after 12-14 years, and, as with Ferula gummosa, marks the end of life of the plant.

The resin is collected from the upper parts of the root, after incising the totally dry plants. The resinous droplets are collected every 5-6 days, with the root being incised 8-10 times within 2 months. The yield is approximately 40-60 g per season.

Adulterations of galbanum with Ferula asafoetida are easy to detect through the high percentage of sulphur-containing compounds.

Table 1: Compounds in galbanum essential oil according to origin (in %)

Origin	Khorassan				Elburz		Zagros
Sample no.	11	13	17	22	24	27	8
a-Pinene	2.60	5.05	3.41	1.48	3.7	1.58	0.97
ß-Pinene	21.36	33.03	21.91	16.54	24.67	46.18	31.33
Myrcene	2.35	2.44	2.78	1.69	1.25	2.36	2.02
a-Phellandrene	1.32	0.63	1.75	0.83	0.16	0.07	0.25
?3-Carene	24.5	12.28	30.94	15.93	0.72	0.38	4.43
Limonene	1.90	1.47	2.7	2.55	0.3	0.39	0.39
ß-Phellandrene	5.95	2.81	6.5	7.7	0.4	0.42	0.71
Ocimene cis-ß	1.41	1.2	1.31	0.95	1.01	0.24	1.53
Terpinolene	1.03	0.31	1.27	0.76	0.03	0.03	0.21
Carvacrol methyl ether	1.48	1.25	0.6	1.45	0.15	0.09	0.07
a-Terpenyl acetate	1.07	0.45	0.81	1.9	1.33	0.96	0.91
Germacrene D	2.7	0.37	0.28	2.76	0.7	0.23	2.22
Bicyclogermacrene	1.35	0.29	2.56	1.76	0.07	0.11	0.28
?-Cadinene	0.6	0.22	0.09	1.14	0.16	0.04	0.56
Germacrene B	3.6	0.6	0.2	4.46	n.d.	n.d.	3.5
Guaiol	1.01	2.15	0.49	1.41	0.95	4.00	7.2
ß-Eudesmol	1.19	2.1	0.88	1.78	1.24	0.71	2.14
Bulnesol	1.11	2.6	1.43	2.75	0.8	5.4	7.28

Dorema ammoniacum D. Don

Dorema ammoniacum D. Don (Apiaceae) yields a resin which is collected from the aerial parts, mainly the inflorescences, as evidenced in the region of Yazd. It is produced as a defence against negative impacts of an insect (Cemambicydae) living in symbiosis with the plant. The insect perforates the sprouts, which causes the flow of a resin oxidizing and solidifying in sunlight. The resin is mainly collected for the Indian market.

In our studies on the resin of *Dorema ammoniacum* Karst., we found two chemotypes so diffently composed that the two types might call for a botanical differentiation. *Dorema ammoniacum* resins yield only small quantities of essential oil. An adulteration of galbanum with the resin of *Dorema ammoniacum* is thus only interesting for the bulk of the resin. It will lead to a decrease in the yield of essential oil. Elemicine, which can be found in quantities up to 40 percent in galbanum from Khorassan, is present in the resin of *Ferula gummosa*, but found only in traces in the essential oil. Its presence in the essential oil in higher quantities is indicative for adulterations with *Dorema ammoniacum.*

Major compounds in the resin of *Dorema ammoniacum* from central Iran with more than 7 percent each are ß-bisabolene and dihydro-a-agarofuran, with both compounds representing only trace compounds in *Ferula gummosa*.

Table 2: Major compounds in *Dorema ammoniacum*, *Ferula asa foetida* and *Ferula gummosa* from Khorassan. *D. ammoniacum* from Yazd shows a distinctly different composition.

Species	*Dorema ammoniacum*		*Ferula asa-foetida*	*Ferula gummosa*
Origin	Yazd	Khorassan	Khorassan	Khorsassan
a-Pinene	0.20	n.d.	0.07	**3.14**
ß-Pinene	0.20	n.d.	0.01	**23.21**
Sabinene	**1.80**	n.d.	n.d.	0.34
?3-Carene	0.50	n.d.	0.35	**20.91**
Limonene + ß-Phellandrene	0.45	1.55	n.d.	**7.90**
Ocimene cis-ß	0.17	n.d.	n.d.	**1.22**
Ocimene trans-ß	0.02	n.d.	n.d.	0.45
a-Ylangene	**1.25**	n.d.	n.d.	0.05
a-Copaene	0.70	n.d.	n.d.	0.09
ß-Elemene	**2.15**	n.d.	n.d.	0.28
ß-Caryophyllene	**2.10**	n.d.	n.d.	0.67
?-Elemene	**2.35**	n.d.	n.d.	0.24
Guaiadiene-6,9	**1.70**	n.d.	n.d.	0.24
ß-Selinene	**2.35**	n.d.	n.d.	0.42
a-Selinene	**4.40**	n.d.	n.d.	n.d.
ß-Bisabolene	**7.15**	n.d.	n.d.	n.d.
Dihydro-a-Agarofuran	**7.80**	n.d.	0.80	0.22
?-Cadinene	**4.25**	n.d.	0.50	0.51
d-Cadinene	**3.15**	n.d.	0.90	0.38
Selina-4,7(11)-diene	**4.00**	n.d.	n.d.	n.d.
Myristicine	n.d.	**3.55**	n.d.	0.58
Pentadecane	n.d.	**4.65**	n.d.	n.d.
Elemicine	n.d.	**40.00**	n.d.	0.46
Heptadecadiene	n.d.	**5.11**	n.d.	n.d.
Heptadecene-8	n.d.	**5.85**	n.d.	n.d.
Myrcene	n.d.	n.d.	0.08	**2.32**
(Z)-1-Propenyl-sec-butyl disulfide	n.d.	n.d.	**4.20**	n.d.
(E)-1-Propenyl-sec-butyl disulfide	n.d.	n.d.	**8.80**	n.d.
(E)-Sec-butyl-butene-2-disulfide	n.d.	n.d.	**1.10**	n.d.
Guaiol	n.d.	n.d.	0.70	1.27
10-Epi-?-eudesmol	n.d.	n.d.	**2.25**	n.d.

Conclusions

Whenever the purity of commercial herbal raw material has to be guaranteed, the concept of traceability becomes absolutely indispensable. As the study of typical adulterants allows to identify typical chemical marker compounds, the quality of regular supplies can be more easily controlled. In addition, the identification of a range of naturally occurring phytochemical compositions in chemotypes of *Ferula gummosa* originating from different regions allows to better respond to specific requirements of the companies using the raw material, and helps to establish specifications of the products.

In the countries where galbanum-containing products are sold, the consumer and the producing companies will profit from a suitable control of the quality of herbal raw material and the detection of adulterations. Perhaps even more important is the stabilizing impact of traceability and quality control on the economic situation in the countries where galbanum is collected, and on the awareness of sustainability in growing and harvesting of *Ferula gummosa*.

References

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