ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 20(1) 2021, 3–14
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2021.1.1
ORIGINAL PAPER
Accepted: 15.11.2019

BIOCHEMICAL CHARACTERIZATION OF FENNEL (*Ferula communis* L.) DIFFERENT PARTS THROUGH THEIR ESSENTIAL OILS, FATTY ACIDS AND PHENOLICS

Fatma Zohra Rahali[1], Myriam Lamine[2], Iness Bettaieb Rebey[1], Wissem Aidi Wannes[1] ✉, Majdi Hammami[1], Sawsen Selmi[1], Ahmed Mliki[2], Ibtissem Hamrouni Sellami[1]

[1] Laboratory of Medicinal and Aromatic Plants, Biotechnology Center of Borj-Cedria, BP 901, Hammam-Lif 2050, Tunisia
[2] Laboratory of Plant Molecular Physiology, Biotechnology Center of Borj-Cedria, BP 901, Hammam-Lif 2050, Tunisia

ABSTRACT

The intention of this study was to compare the different parts of Tunisian *Ferula communis* via their fatty acids, essential oils and phenolic compounds. Results showed that the lipid fraction of fruits and leaves was characterized by the predominance of oleic acid. Erucic and linoleic acids were the most abundant in stems while linoleic and palmitic acids in flowers. *F. communis* essential oils were defined by four chemotypes, namely isoshyobunone/6-tert-butyl-4-methylcoumarin in stems; α-eudesmol/caryophyllene oxide in leaves; caryophyllene/myrcene in flowers and α-gurjunene/hexadecanoic acid in fruits. So, oxygenated sesquiterpenes and sesquiterpene hydrocarbons represented the major classes of stem essential oil. Monoterpene hydrocarbons were the predominant classes of leaves and flowers. Fruit essential oil was predominated by sesquiterpene hydrocarbons. Resorcinol and ferulic acid were the main phenolic compounds in flowers but chlorogenic and ferulic acides in leaves. Stems were rich in ferulic acid and quercetin while leaves in coumarin and tannic acid. Besides to the high variability among *F. communis* parts, this plant contained high amounts of bioactive compounds with various health benefits attributed to their antioxidant potential.

Key words: *Ferula communis*, essential oil, fatty acids, polyphenols, endogenous variability

INTRODUCTION

The genus *Ferula* has broadly been used in traditional medicine for a varied range of disorders and its pharmacological effects are well documented in both human and veterinary practices [Singh et al. 1988]. However, several *Ferula* species are characterized by their toxicity to humans and animals [Marchi et al. 2003]. The therapeutic use of *Ferula* genus has been proven by several researchers as anti-cancer [Saleem et al. 2001], anti-diabetic [Iranshahi and Iranshahi 2011], anti-bacterial, anti-ulcerative and anti-inflammatory effects [Li et al. 2015a]. These biological properties are principally owing to the abundance of active phytochemicals, namely sesquiterpene derivatives [Li et al. 2015b], organic acid glycosides, steroidal esters and sulfur-containing compounds [Iranshahi 2012].

Ferula communis L. (Giant fennel) is a latex-containing perennial odoriferous plant, 1–2.5 m high with dense roots. Its cylindrical peduncle is green, striated, with slimy exudate. The branches, 8–10 cm long, are alternate (inferior) or opposite (superior). The leaves are glabrous, with a large sheath. The inflorescence is attached on the terminal part of the peduncle. The plant, despite the name, is not a type of fennel proper belonging to the other genus *Foeniculum*. The name of

✉ aidiwissem@yahoo.fr

© Copyright by Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie

the phenolic compound ferulic acid, which can be isolated from giant fennel, is derived from the Latin name of the plant [Akaberi et al. 2015]. Hence, the fruit and stem of *F. communis* have been reported to be rich in chlorogenic acid and ferulic acid while the flower was rich in resorcinol and ferulic acid [Rahali et al. 2018]. The phenolic acids chlorogenic and ferulic are also abundant in other natural sources as coffee, tea, cereals, many fruits and vegetables. In general, polyphenols exhibit strong antioxidant properties against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [Graf et al. 2005]. Additionally, Karakaya et al. [2019] has been observed that hexane fraction of roots has noticeable antioxidant-anticholinesterase activities.

The essential oils of *Ferula* genus have an important role in the anti-bacterial, anti-microbial, anti-viral and anti-fungal activities as well as in some other particular medicinal effects [Bakkali et al. 2008, Maggie et al. 2009]. Ferrari et al. [2005] found that the main component of leaf essential oil from Corsican *F. communis* was myrcene. Marongiu et al. [2005] noted that α- and β-gurjunene were the predominant components of flower heads from Sardinian *F. communis* obtained by hydrodistillation and supercritical fluid extraction. Rubiolo et al. [2006] studied the volatile fraction obtained from aerial parts of the two chemotypes growing in Sardinia, and detected that aristolene and farnesol were the major components of aerial part volatile fraction from the poisonous *F. communis* chemotype, and allohedycaryol in the non-poisonous one. Rahali et al. [2016] reported that three *F. communis* chemotypes could be defined in leaf essential oil from Tunisia which were α-eudesmol/β-eudesmol/γ-terpinene; α-eudesmol/α-pinene/caryophyllene oxide and chamazulene/α-humulene chemotypes. Finally, Nguir et al. [2016] found that the essential oil of Tunisian *F. communis* stem was rich in β-eudesmol, δ-eudesmol, and α-eudesmol. Concerning the flower, it was rich in camphor, α-pinene and β-eudesmol while the root in dillapiole, guaiol and spathulenol.

The determination of fatty acid composition is very important to assess the overall nutritional quality of a food. Furthermore, the *Apiaceae* plants are identified by their richness in petroselinic acid constituting a good material for food, cosmetic, and pharmaceutical industries [Baird and Preskett 2013]. However, no report was found concerning the fatty acid composition of *F. communis* different parts.

Thus, since the multiple uses of each of the plant parts of *F. communis*, this work aimed to investigate essential oil, fatty acid and phenolic composition of stems, leaves, flowers and fruits of this valuable plant. To the best of our knowledge, this work represented the first scientific report concerning lipid fraction and the evaluation of a detailed metabolite profiling of *F. communis*.

MATERIALS AND METHODS

Botanical material. Giant fennel parts were wildly gathered during May 2014 in Rades, Northeast Tunisia. A voucher specimen (P.l.08003) was identified by Professor Smaoui and deposited in Herbarium of the Laboratory of Bioactive Substances (Biotechnology Center of Borj Cedria).

Essential oil isolation, analysis and identification. The essential oil extraction of 300 g of dried samples from leaves, flowers, fruits and stems was performed by the hydrodistillation using a Clevenger-type apparatus for 120 min. The obtained essential oils were kept at –20°C in darkness.

The essential oil analysis was performed using Gas Chromatography-Mass Spectrometry (GC-MS) using an Agilent 5975C mass spectrometer with electron impact ionization (70 eV) coupled with an Agilent 7890 A series II gas chromatograph. An HP-5MS capillary column (30 m · 0.25 mm coated with 5% phenyl methyl silicone, and 95% dimethyl polysiloxane, 0.25 μm film thickness) was used. The program used was isotherm at 70°C, followed by 50–240°C at a rate of 5°C min, then held at 240°C for 10 min. Essentials oil volatile compounds were identified by comparing their retention indexes (RI) related to (C9–C18) n-alkanes with those of authentic compounds (Analytical reagents, LabScan, Ltd, Dublin, Ireland) available in literature and in our laboratory and by matching their mass spectra fragmentation patterns with corresponding data stored in the mass spectra library of the GC–MS data system (NIST) and other published mass spectra [Adams 2001]. Relative percentage amounts of the identified compounds were obtained from the electronic integration of the FID peak area. Analyses were performed in triplicate.

Total lipid and fatty acid determination. Chloroform/methanol mixture (2 : 1, v/v) was used for lipid extraction. 1 g of *F. communis* organs were kept in boiling water for 5 min and then ground manually using a mortar and pestle. After washing by fixation water, the organic layer containing lipids was recovered and dried under a nitrogen stream [Bligh and Deyer 1959].

According to Cecchi and Traverso [1985], total fatty acids (TFAs) of total lipids were transmethylated using sodium methoxide solution – 3% in methanol (Sigma–Aldrich, Analytical reagent, LabScan Ltd., Dublin, Ireland).

FAMEs were analyzed by gas chromatography using a Hewlett-Packard 6890 gas chromatograph series II (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. The initial oven temperature was held at 150°C for 1 min, increased at a rate of 15°C/min to 200°C, and for 3 min and finally ramped at 2°C/min to 242°C. The detector and injector temperatures were set at 275°C and 250°C, respectively. Results presented are means of three repetitions ± standard error.

FAMEs were identified by comparison of their retention times with those of pure reference standards. Relative percentage amounts of the identified FAMEs were obtained from the electronic integration of the FID peak area. Analyses were performed in triplicate.

Phenolic determination. 3 g of each powder sample was separately extracted by shaking with 30 ml of pure methanol for 30 min. The extracts were then kept for 24 h at 4°C, filtered through a Whatman filter paper (No. 4), dried under vacuum and stored at 4°C until their analysis [Mau et al. 2001].

The phenolic compound analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP-HPLC) coupled with a UV–Vis multi-wavelength detector and equipped with a 250 × 4.6 mm, 4 µm Hypersil ODS C18 reversed phase column kept at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 mL/min. The gradient program was as follows: 15% A/85% B 0–12 min, 40% A/60% B 12–14 min, 60% A/40% B 14–18 min, 80% A/20% B 18–20 min, 90% A/10% B 20–24 min, 100% A 24–28 min. The injection volume was 20 µl and peaks were monitored at 280 nm. Samples were filtered through a 0.45 µm membrane filter before injection. Peak identification was made by congruent retention times compared to standards. Relative percentage amounts of the identified compounds were automatically measured by an integrator of HPLC instrument. Analyses were performed in triplicate.

Statistical analysis

Experiments were shown as means ± standard deviation of the mean from three replicates (n = 3). The *p*-value <0.05 was used to denote significant differences between mean values determined using one-way analysis of variance (ANOVA) and the Duncan's multiple range test done using the assistance of IBM® SPSS® Statistics program 20.0 (SPSS Inc., Chicago, IL, USA). Multivariate analysis including principal component analysis (PCA) and hierarchical clustering analysis was performed for metabolite data using statistical analysis software (XLSTAT 18.0).

RESULTS AND DISCUSSION

Essential oil yield. The yields of light pale yellow-essential oils extracted from different *F. communis* parts (stems, leaves, flowers and fruits) are reported in Figure 1. The highest essential oil yield was detected in leaves (0.22%), followed by flowers (0.10%), fruits (0.08%) and stems (0.07%). These essential oil yields exceeded by far those determined by Maggi et al. [Maggi et al. 2016] in Italian *F. communis* with 0.13% in flowers, 0.06% in leaves, 0.03% in fruits and 0.02% in roots. Nguir et al. [2016] reported that Tuni-

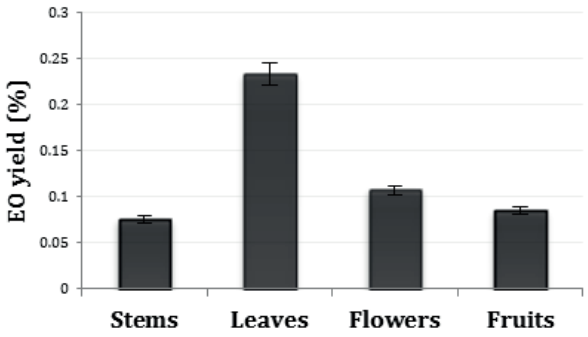

Fig. 1. Yields (% on dry weight basis) of *F. communis* parts essential oils

sian *F. communis* had an essential oil yield of 0.18% in flowers, 0.15% in stems, 0.11% in leaves and 0.02% in roots. So, there was a significant variation of essential oil yields from *F. communis* depending on both organ and origin which could be due to environmental and genetic factors. In our study, *F. communis* leaves were the potential source of essential oil production, as previously demonstrated for other *Ferula* species such as *F. vesceritensis* [Bouratoua et al. 2014], *F. oopoda* and *F. badghysi* [Akhgar et al. 2011].

Essential oil profile. Chromatographic analysis of the essential oils from different *F. communis* parts allowed the identification of 64 compounds from stems, 42 compounds from leaves, 72 compounds from flowers and 42 from fruits (Tab. 1). Oxygenated sesquiterpenes (35.11%) and sesquiterpene hydrocarbons (14.37%) represented the major classes of stem essential oil. Monoterpene hydrocarbons were the predominant classes of leaves and flowers (34.31%, 26.36%, respectively). Fruit essential oil was predominated by sesquiterpene hydrocarbons (28.82%). The main constituents of stem essential oil were isoshyobunone (18.3%), 6-tert-butyl-4-methylcoumarin (7.95%) and β-bisabolene (5.94%). The leaf essential oil was characterized by the predominance of α-eudesmol (12.3%), caryophyllene oxide (5.47%), ɣ-terpinene (5%), α-pinene (5%) and γ-cadinene (5%). Flower essential oil was rich in caryophyllene (15.12%), myrcene (10.28%), α-eudesmol (9.42%) and α-pinene (8.26%). However, hexadecanoic acid (16.45%) and α-gurjunene (10.45%) were the predominant compounds of fruit essential oil. From this qualitative variation, it appeared that the chemical composition of *F. communis* essential oil was organ-dependent. Indeed, each organ was characterized by an unique volatile profile. Such variability in the essential oil composition between the different *F. communis* parts was previously elucidated from different localities. In Greece, α-eudesmol (12.6%) and β-eudesmol (9.7%) were abundant in leaves, α-pinene (35.2%) and β-pinene (13.6%) in fruits as well as γ-curcumene (14%), *ar*-curcumene (8.5%) and γ-terpinene (10.8%) in flowers [Manolakou et al. 2013]. In Corsica, Ferrari et al. [2005] reported that *F. communis* leaf oil was characterized by the presence of myrcene (53.5%) and aristolene (8.5%). Sardinian *F. communis* flower oil was rich in α-gurjunene (40.7%) and β-gurjunene (7.1%) as mentioned by Marongiu et al. [2005]. In Algeria, the oil of *F. communis* aerial parts had myrcene (52.5%), α-pinene (20.9%) and β-phyllendrene (7.7%) as the main volatile compounds [Chibani et al. 2011]. Our results corroborated these earlier studies showing that *F. communis* presented an intraspecific variability, with different chemotypes as a result of environment and genetic selection [Maggi et al. 2016].

The principal component analysis (PCA) was performed to determine possible relationships between the different organs studied based on the composition of their essential oils. As shown in Figure 2, *F. communis* stems were significantly different from the other three organs. The model of all samples explained 78.01% of the principal components, with the principal component 1 (PC1) interpreting 46.03% and principal component 2 (PC2) interpreting 29.52%. According to PC1, the scatter plot indicated that the stems were remarkably separated from leaves, flowers and fruits. Besides, stems were clustered by negative scores on PC1, nevertheless fruits presented positive scores on PC1, suggesting that fruits and stems were completely dissimilar in metabolic patterns from the other parts. Stems and flowers and leaves were further segregated on PC2, which indicated that the metabolic pattern was also different.

Total lipid content. The total lipid yield of different *F. communis* organs (flowers, fruits, stems and leaves) is shown in Figure 3. *F. communis* fruits exhibited the highest total lipid yield (0.18%), followed by leaves (0.10%), flowers (0.06%) and stems (0.05%). In general, seeds and fruits were the plant parts of fat and lipid accumulation as mentioned by Caprioli et al. [2014] in the case of *Smyrnium olusatrum* belonging to *Apiaceae* family with fat content of 7.1% in ripe fruits, 0.3% in roots, 0.2% in leaves and 0.1% in basal leaves. To our knowledge, there was no previous work dealing with the total lipid content and the fatty acid composition of the different *F. communis* organs. Concerning the other *Ferula* species and as for several *Apiaceous* plants, numerous studies had focused in seed oils such as *F. asafetida* [1989], *F. Jaeschkeana* [Seemal et al. 1988] and *F. parva* [Ghafoor et al. 2019]. Additionally, an analysis of *F. asafoetida* roots showed the presence of fat yielding 1.1% [Mahendra and Bisht 2012].

Fatty acid profile. Fatty acid composition of different *F. communis* parts (fruits, flowers, stems and

Table 1. Chemical composition of *F. communis* essential oils from different parts

Compounds	RI	Stems	Leaves	Flowers	Fruits
α-pinene	939	2.25	5.00	8.26	2.04
β-pinene	980	1.93	–	1.73	1.63
ɣ-terpinene	1063	–	5.00	–	–
α-thujene	928	–	–	–	3.00
δ-3-carene	1011	–	–	2.29	–
O-cymene	–	–	–	1.43	–
Myrcene	991	–	3.00	10.28	1.97
p-mentha-1.5-dien-8-ol	–	–	–	6.00	–
β-cubebene	1377	–	–	2.17	–
Caryophyllene	1419	–	3.76	15.12	–
ɣ-curcumene	1487	–	–	3.82	–
ar-curcumene	1484	–	5.00	–	–
Valencene	1492	–	–	1.75	–
β-bisabolene	1509	5.94	2.00	2.27	1.23
ɣ-eudesmol	1629	–	–	3.19	–
α-eudesmol	1639	–	12.30	9.42	–
β-ocimene	1040	–	–	–	2.25
Myrtenal	1193	–	–	–	2.26
α- humulene	1455	–	2.45	–	1.23
α-copaene	1376	–	–	–	2.08
ɤ-cadinene	1524	–	5.00	–	1.53
β-himachalene	1499	–	–	–	1.14
α-gurjunene	1409	–	–	–	10.45
Di-epi-.alpha.-cedrene	1382	–	–	–	2.24
α- calacorene	1548	–	–	–	1.69
(+)-epi-bicyclosesquiphellandrene	1482	–	–	–	1.14
Himachalol	1647	–	–	–	1.13
α-cadinol	1653	–	–	–	4.49
Hexadecanoic acid	1984	–	–	–	16.45
9.12-octadecadienoic acid	2130	–	–	–	2.57
D-limonene	1031	2.19	–	–	–
Germacrene B	1556	–	1.29	–	–
Caryophyllene oxide	1581	4.38	5.47	–	–
α- himachalene	1447	1.56	–	–	–
β-chamigrene	1475	2.78	–	–	–
Calamenene	1521	2.69	–	–	–
δ-cadinol	1636	1.00	–	–	–
Cedrenol	1604	4.16	–	–	–
α- acorenol	1629	3.92	–	–	–
Isoshybunone	1482	18.30	–	–	–
Alcohols		0.80	13.50	0.00	0.00
Aldehydes		–	0.58	0.55	0.00
Alkanes		–	0.83	0.32	0.00
Fatty acids		–	0.45	0.58	20.36
Phenols		–	2.74	0.24	0.00
Monoterpenes		5.08	34.31	26.36	8.77
Oxygenated monoterpenes		5.04	9.07	9.50	3.74
Sesquiterpènes		14.37	15.05	28.59	28.82
Oxygenated sesquiterpenes		35.11	7.27	19.41	6.53
Others		2.62	7.18	10.25	11.26

RI: retention indices relative to n-alkanes (alkane standard solution C8–C40 40147-U Supelco) on apolar column HP-5MS

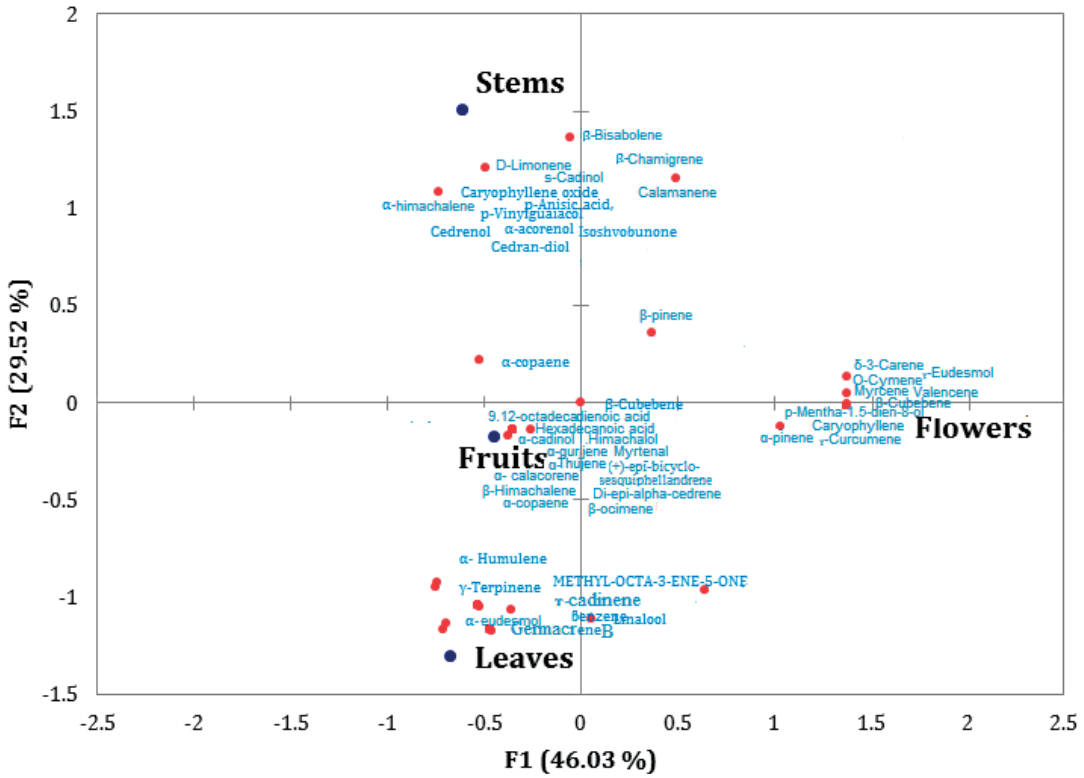

Fig. 2. Principal component analysis performed on volatile composition

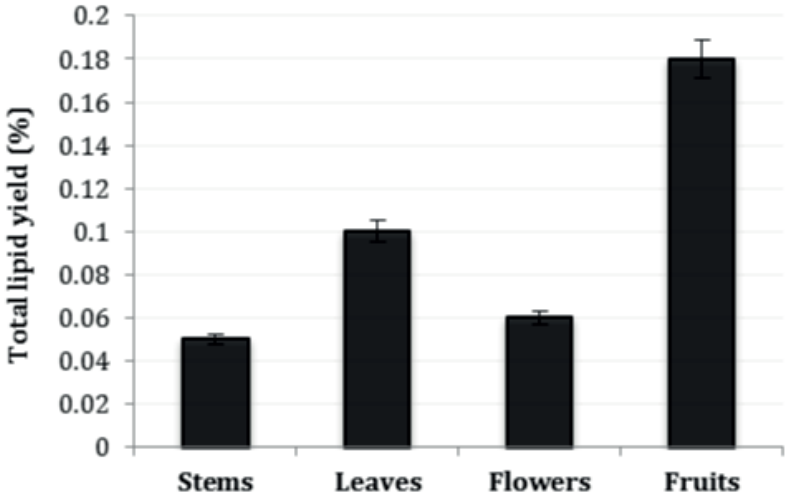

Fig. 3. Total lipid yields (% on dry weight basis) of *F. communis* parts

leaves) is reported in Table 2. Flowers and leaves exhibited the highest amounts of polyunsaturated fatty acids with 36.96 and 36.05%, respectively. The highest amount of monounsaturated fatty acids was detected in fruits (10.41%) and flowers (8.14%), while the lowest amount was detected in the leaves (6.21%). Saturated fatty acids were the predominant class of fruits (57.87%), flowers (50.96%), stems (42.11%) and leaves (26.54%).

Among 12 identified fatty acids, erucic (C22:1; 18.28%), linoleic (C18:2; 11.43%), palmitic (C16:0; 11.38%) and myristic (C14:0; 11.11%) acids were the major ones in *F. communis* stems. Nineteen fatty acids were identified in *F. communis* leaves, where oleic acid (C18:1) was the predominant fatty acid (26.81%), followed by α-linolenic (C18:3n3; 13.14%), palmitic (C16:0; 12.64%), octadecatetraenoic (C18:4; 11.81%) and linoleic (C18:2; 10.07%) acids. Nineteen fatty acids found in *F. communis* flowers were characterized by the abundance of linoleic acid (C18:2; 28.22%), followed by palmitic (C16:0; 21.88%), α-linolenic (C18:3n3; 16.55%) and oleic (C18:1; 6.48%) acids. The fruits had seventeen fatty acids with the predominance of oleic acid (C18:1; 59.1%), followed by heptadecanoic (C17:0; 22.39%) and palmic (C16:0; 7.16%) acids.

For a better clarification of the fatty acid qualitative variability from the various *F. communis* organs, a principal component analysis (PCA) based on the fatty acid composition was carried out. As shown in Figure 4, fruits were significantly different from the other organs. The model of all samples explained 75.00% of the principal components, with the principal component 1 (PC1) interpreting 46.89% and

Table 2. Fatty acid (FA) composition (%), of *F. communis* different parts

Compounds	RT	Stems	Leaves	Flowers	Fruits
C08:0 caprilic acid	3.75	1.91	0.17	0.29	1.66
C10:0 capric acid	3.98	2.43	0.19	0.59	0.94
C12:0 lauric acid	4.25	–	0.17	0.51	0.36
C14:0 myristic acid	4.63	11.11	2.27	4.42	0.14
C15:0 pentadecanoic acid	5.11	5.2	6.66	1.29	0.29
C16:0 palmitic acid	5.77	11.38	12.64	21.88	7.16
C16:1 palmitoleic acid	6.24	–	0.16	0.18	0.18
C16:3 hexadecatrienoic acid	6.75	5.51	1.03	0.39	0.39
C17:0 heptadecanoic acid	7.01	–	1.59	1.59	22.39
C17:1 heptadecenoic acid	7.55	–	8.21	3.33	–
C18:0 stearic acid	7.72	10.52	2.03	2.13	1.44
C18:1 oleic acid	8.41	–	26.81	6.48	59.1
C18:2 linoleic acid	9.59	11.43	10.07	28.22	2.36
C18:3n3 α-linolenic acid	11.08	5.36	13.14	16.55	0.13
C18:4 α-parinaric acid	11.27	6.95	11.81	1.75	–
C20:0 arachidic acid	11.54	–	0.58	0.47	1.69
C20:1 gadoleic acid	11.75	–	0.13	0.42	0.77
C22:0 behenic acid	12.11	9.91	0.25	1.43	0.87
C22:1 erucic acid	12.55	18.28	2.07	6.17	0.11
SFA		42.11	26.54	50.96	57.87
MUFA		7.06	6.21	8.14	10.41
PUFA		34.22	36.05	36.96	3.53

RT: retention time (min); SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid

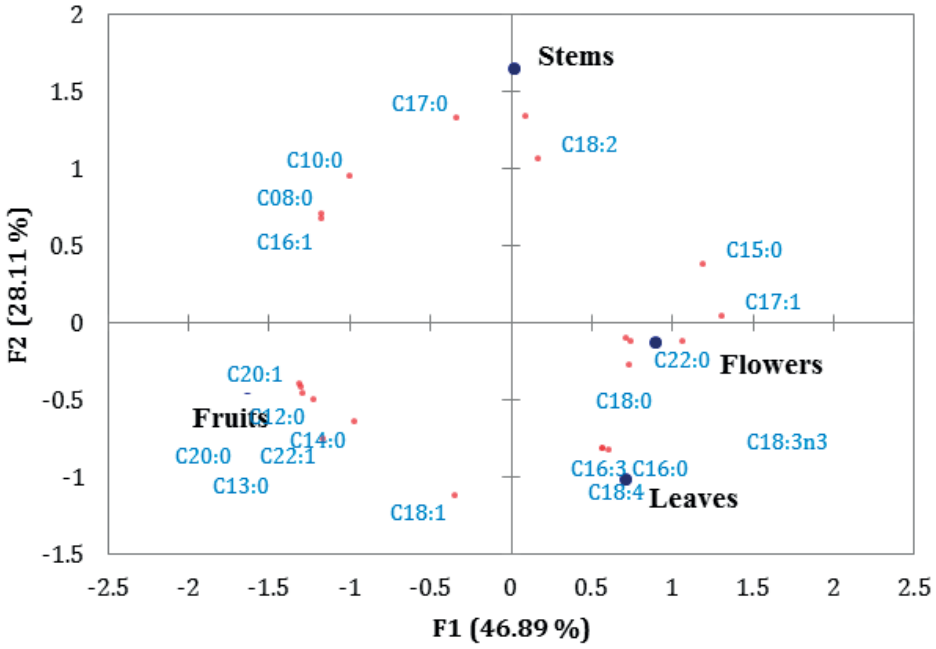

Fig. 4. Principal component analysis performed on fatty acid composition

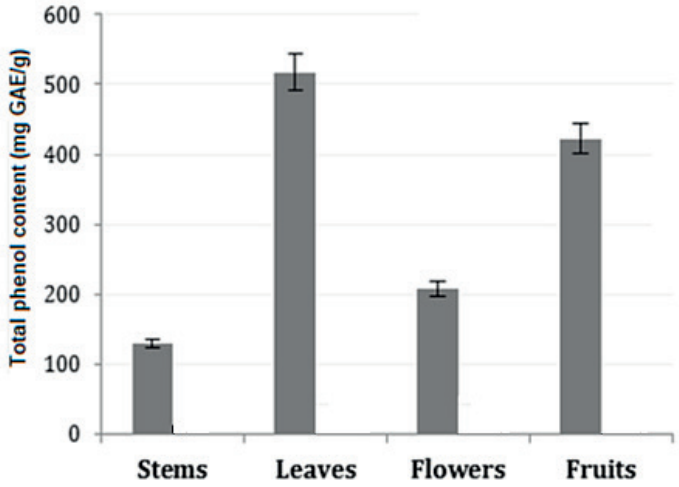

Fig. 5. Total phenolic and flavonoids contents of different parts of *F. communis*. Total phenolic compounds were expressed by mg GAE/g DW. Total flavonoid compounds were expressed by mg QE/g DW. Values are represented as mean ±SD of triplicates. GAE: gallic acid aquivalents, QE: quercetin equivalents

principal component 2 (PC2) interpreting 28.11%. According to PC1, the scatter plot indicated that the stems were remarkably separated from leaves, flowers and fruits. Fruits were clustered by negative scores on PC1, nevertheless flowers, leaves and stems presented positive scores on PC1, suggesting that fruits and the other organs were completely dissimilar in fatty acid profile. *F. communis* fruits and leaves exhibited the most similar lipid composition.

Total polyphenol content. From Figure 5, total phenolic contents of different *F. communis* parts were clearly organ-dependent varying from 129.28 mg EGA/g DW in stems, 207.21 mg EGA/g DW in flowers, 422 mg EGA/g DW in fruits, to 517.07 mg EGA/g DW in leaves. Thus, *F. communis* aerial parts constituted an interesting source of polyphenols. Phenolic content variability between *F. communis* organs had been previously reported by Rahali et al. [2018] who found that fruits (422 mg EGA/g DW) contained more phenols than flowers (207.21 mg EGA/g DW) and stems (129.28 mg EGA/g DW). In the case of *F. gummosa*, Nabavi et al. [2010] found that flowers (20.8 mg EGA/g MS DW) had higher phenolic content than leaves (18.5 mg EGA/g MS DW) and stems (12.9 mg EGA/g MS DW). This endogenous variability in plants could be probably due to the complex nature of phenolic compounds and the various physiological roles which they performed in each organ.

Phenolic compound identification. A detailed analysis of qualitative polyphenol variability from different *F. communis* parts was performed by an RP-HPLC analysis (Tab. 3). Nine phenolic compounds were successfully identified in stems where chlorogenic acid was the major one (10.89%). This organ was also characterized by the presence of ferulic acid (6.71%) and quercetine (2.29%). In leaves, 13 compounds were identified. The most abundant ones were coumarin (20.2%), tannic acid (13.75%) and resorcinol (13.62%). Nine phenolic compounds were found in *F. communis* flowers with the abundance of resorcinol (35.46%), ferulic acid (15.26%) and syringic acid (10.71%). *F. communis* fruits contained ten phenolic compounds with the predominance of chlorogenic acid (18.38%), ferulic acid (10.41%) and coumarin (6.35%).

Similar results were obtained by Rahali et al. [2018] concerning the phenolic composition of *F. communis* fruits, stems and flowers. However, no data was available concerning the phenolic composition of *F. communis* leaves. In fact, it could be deduced that the nature of phenolic compounds was very heterogeneous

Table 3. Phenolic compound composition (%) of different *F. communis* parts

Compounds	RT	Stems	Leaves	Flowers	Fruits
Tannic acid	3.19	1.99	13.75	1.62	0.6
Gallic acid	5.29	1.55	10.33	1.7	0.76
Syringic acid	10.23	–	3.22	10.71	1.44
Ferulic acid	12.70	6.71	5.76	15.26	10.41
Chlorogenic acid	14.34	10.89	4.22	–	18.38
Hydroxycinnamic acid	14.90	–	2.02	–	–
Catechin hydrate	15.11	1.02	–	3.11	1.44
Catechin	15.55	0.74	–	–	2.36
Resorcinol	16.80	1.94	13.62	35.46	–
Coumarin	18.23	–	20.2	7.11	6.35
Quercetin	18.42	2.29	6.96	3.25	1.42
Flavone	20.20	2.09	–	2.62	1.79
Hyperoside	21.33	–	3.2	–	–
Rosmarinic acid	21.62	–	0.3	–	–
Oleuropeine	21.91	–	0.1	–	–
Kaempferol3-O-rutinoside	22.53	–	1.5	–	–

RT: retention time (min)

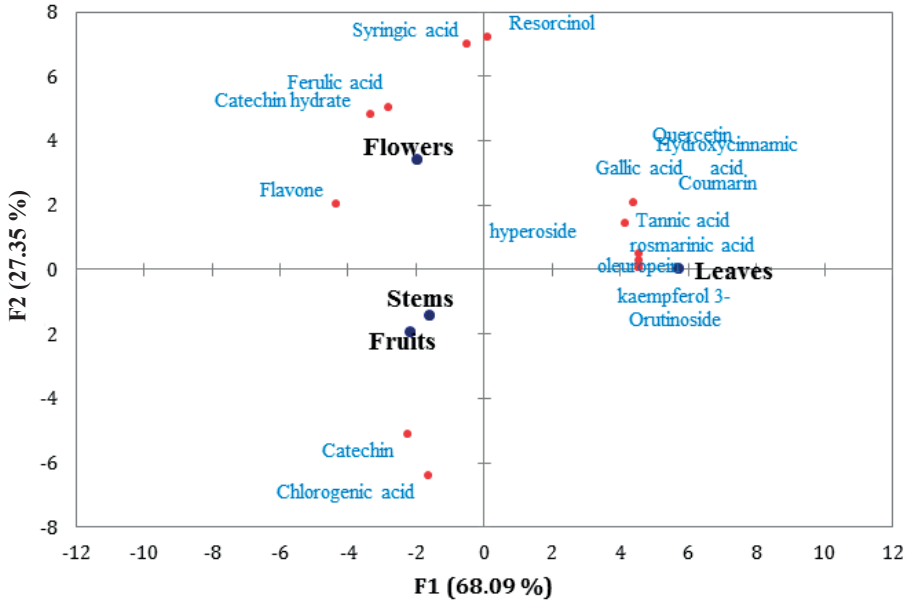

Fig. 6. Principal component analysis performed on phenolic composition

between the four organs. This specific distribution of bioactive compounds in different *F. communis* parts was probably attributed to the important need in these so-called "stress" compounds to fight against various biotic and/or abiotic stresses (ultraviolet radiation, herbivores, etc.) as reported by Macheix et al. [2005].

The principal component analysis revealed the existence of three distinct groups: stems and fruits have the most similar profile, while flowers and leaves had the most distinct ones. PCA results showed that the first two principal axes represented 95.44% of the total variance, thus, the first axis contributed with 68.09% of the total variation whereas the second axis with 27.35% (Fig. 6). The two-dimensional axial system of the PCA identified three groups.

CONCLUSION

Based on our findings, the phytochemical investigation of *F. communis* different parts (leaves, flowers, fruits and stems) showed the presence of distinct patterns of primary (fatty acids) and secondary metabolites (essential oils and phenolic compounds).

ACKNOWLEDGMENT

This work was financially supported by the Tunisian Ministry of Higher Education and Scientific Research.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

REFERENCES

Adams, R.P. (2001). Identification of essential oil components by gas chromatography/ quadrupole mass spectroscopy. Allured: Carol Stream, IL.

Akaberi, M., Iranshahy, M., Iranshahi, M. (2015). Review of the traditional uses, phytochemistry, pharmacology and toxicology of giant fennel (*Ferula communis* L. subsp. *communis*). Iran J. Basic Med. Sci., 18, 1050–1062.

Akhgar, M.R., Moradalizadeh, M., Faghihi-Zarandi, A., Rajaei, P. (2011). Chemical composition of the essential oils of *Ferula oopoda* (Boiss. & Buhse) Boiss. and *Ferula badghysi* (Korovin.) from Iran. J. Essent. Oil-Bear Pl., 14, 297–301.

Baird, M.S., Preskett, D. (2013). Method of obtaining a solid component rich in a petroselinic compound. European patent: EP 2, 235, 150 B1.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2008). Biological effects of essential oils a review. Food Chem. Toxicol., 46, 446–475.

Bligh, E.G., Deyer, W.J. (1959). A rapid method of total lipid extraction and purification. Canad. J. Biochem. Physiol., 37, 911–917.

Bouratoua, A., Ferhat, M., Kabouche, A., Laggoune, S., Touzani, R., Kabouche, Z. (2014). Comparative compositions of essential oils of Ferula., 5, 1214–1217.

Caprioli, G., Fiorini, D., Maggi, F., Marangoni, M., Papa, F., Vittori, S., Sagratini, G. (2014). Ascorbic acid content, fatty acid composition and nutritional value of the neglected vegetable Alexanders (*Smyrnium olusatrum* L., *Apiaceae*). J. Food Comp. Anal., 35, 30–36.

Cecchi, E., Traverso, P.G. (1985). Biogas dalla frazione organica di rifiuti solidi urbani e fanghi di supero – Studio Preliminare [Biogas from the organic fraction of municipal solid waste and sewage sludge – Preliminary Study]. Chim. Ind., 67, 609–616.

Chibani, S., Berhail-Boudouda, H., Kabouche, A., Aburjai, T., Kabouche, Z. (2011). Analysis of the essential oil of *Ferula communis* L. from Constantine (Algeria). Int. J. Med. Arom. Pl., 1, 41–44.

Ferrari B., Tomi F., Casanova, J. (2005). Composition and chemical variability of *Ferula communis* essential oil from Corsica. Flav. Fragr. J., 20, 180–185.

Ghafoor, K., Doğu, S., Ahmed, I.A.M., Fadimu, G.J., Geçgel, Ü., Al Juhaimi, F., Babiker E.E., Özcan, M.M. (2019). Effect of some plant species on fatty acid composition and mineral contents of *Ferulago, Prangos, Ferula*, and *Marrubium* seed and oils. J. Food Process. Preserv., E13939.

Graf, B.A., Milbury, P.E., Blumberg, J.B. (2005). Flavonols, flavonones, flavanones and human health: Epidemological evidence. J. Med. Food, 8, 281–290.

Iranshahi, M. (2012). A review of volatile sulfur-containing compounds from terrestrial plants: biosynthesis, distribution and analytical methods. J. Essent. Oil Res., 24, 393–434.

Iranshahi, M., Iranshahi, M. (2011). Traditional uses, phytochemistry and pharmacology of *asafoetida* (*Ferula assafoetida* oleo-gum-resin) – a review. J. Ethnopharmacol., 134, 1–10.

Karakaya, S., Koca, M., Sytar, O., Duman, H. (2019). Determination of natural phenolic compounds of *Ferula longipedunculata* Peşmen and assessment their antioxidant and anticholinesterase potentials. Nat. Prod. Res., 29, 1–3.

Li, G., Wang, J., Li, X., Cao, L., Lv, N., Chen, G., Zhu, J., Si, J. (2015a). Two new sesquiterpene coumarins from the seeds of *Ferula sinkiangensis*. Phytochem. Lett., 13, 123–126.

Li, G.Z., Li, X.J., Cao, L., Zhang, L.J., Shen, L.G., Zhu, J., Wang, J.C., Si, J.Y. (2015b). Sesquiterpene coumarins from seeds of *Ferula sinkiangensis*. Fitoterapia, 103, 222–226.

Macheix, J.J., Fleuriet, A., Jay-Allemand, C. (2005). Les composés phénoliques des végétaux, un exemple de métabolites secondaires d'importance économique [Phenolic compounds in plants, an example of the economic importance of secondary metabolites]. Presses Polytechniques et Univesitaires Romandes. Lausanne, pp. 192.

Maggi, F., Cecchini, C., Cresci, A., Coman, M.M., Tirillini, B., Sagratini, G., Papa, F. (2009). Chemical composition and antimicrobial activity of the essential oil from *Ferula glauca* L. (*F. communis* L. subsp. glauca) growing in Marche (central Italy). Fitoteraphy, 80, 68–72.

Maggi, F., Papa, F., Dall'Acqua, S., Nicoletti, M. (2016). Chemical analysis of essential oils from different parts of *Ferula communis* L. growing in central Italy. Nat. Prod. Res., 30, 806–813.

Mahendra, P., Bisht, S. (2012). *Ferula asafoetida*: Traditional uses and pharmacological activity. Pharmacog. Rev., 6, 141–146.

Manolakou, S., Tzakou, O., Yannitsaros, A. (2013). Volatile constituents of *Ferula communis* L. subsp. communis growing spontaneously in Greece. Rec. Nat. Prod. 7, 54–58.

Marchi, A., Appendino, G., Pirisi, I., Ballero, M., Loi, M.C. (2003). Genetic differentiation of two distinct chemotypes of *Ferula communis* (*Apiaceae*) in Sardinia (Italy). Biochem. Syst. Ecol., 31, 1397–1408.

Marongiu, B., Piras, A., Porcedda, S. (2005). Comparative analysis of the oil and supercritical CO_2 extract of *Ferula communis* L. J. Essent. Oil Res.,17, 150–152.

Mau, J.L., Chao, G.R., Wu, K.T. (2001). Antioxidant properties of methanolic extracts from several ear mushrooms. J. Agric. Food Chem., 49, 5461–5467.

Nabavi, S.F., Ebrahimzadeh, M.A., Nabavi, S.M., Eslami, B. (2010). Antioxidant activity of flower, stem and leaf extracts of *Ferula gummosa* Boiss. Grasas Aceites, 61, 244–250.

Nguir, A., Mabrouk, H., Douki, W., Ben Ismail, M., Ben Jannet, H., Flamini, G., Hamza, M.A. (2016). Chemical composition and bioactivities of the essential oil from different organs of *Ferula communis* L. growing in Tunisia. Med. Chem. Res., 25, 515–525.

Rahali, F.Z., Kefi, S., Bettaieb Rebey, I., Hamdaoui, G., Tabart, G., Kevers, C., Franck, T., Mouithys-Mickalad,

A., Hamrouni Sellemi, I. (2018). Phytochemical composition and antioxidant activities of different aerial parts extracts of *Ferula communis* L. Plant. Biosys., 153, 213–221.

Rahali, F.Z., Lamine, M., Gargouri, M., Bettaieb Rebey, I., Hammami, M., Hamrouni Sellemi, I. (2016). Metabolite profiles of essential oils and molecular markers analysis to explore the biodiversity of *Ferula communis*: Towards conservation of the endemic giant fennel. Phytochem., 124, 58–67.

Rubiolo, P., Matteodo, M., Riccio, G., Ballero, M., Christen, P., Fleury-Souverain, S., Veuthey, J.L., Bicchi, C. (2006). Analytical discrimination of poisonous and nonpoisonous chemotypes of giant fennel (*Ferula communis* L.) through their biologically active and volatile fractions. J. Agric. Food Chem., 54, 7556–7563.

Saleem, M., Alam, A., Sultana S. (2001). *Asafoetida* inhibits early events of carcinogenesis: a chemopreventive study. Life Sci., 68, 1913–1921.

Seemal, J.T., Saleem, M., Ahmad, M., Waheed, I., Bhatty, M.K. (1988). Lipid composition of *Ferula jaeschkeana*: Presence of an odd fatty acid. Pak. J. Sci. Ind. Res., 31, 626–628.

Singh, M.M., Agnihotri, A., Garg, S.N., Agarwal, S.K., Gupta, D.N., Keshri, G., Kamboj, V.P. (1988). Antifertility and hormonal properties of certain carotane sesquiterpenes of *Ferula jaeschkeana*. Planta Med., 54, 492–494.