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Chemical Compositions and Antibacterial Activity of Four Essential Oils from *Ferula vesceritensis* Coss. & Dur. Against Clinical Isolated and Food-Borne Pathogens

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Abstract: The aims of this study were to compare the chemical composition and antibacterial activity of four hydrodistilled oils of *Ferula vesceritensis* Coss. & Dur. Essential oils were obtained from the hydrodistillation of fresh flowers (FF), dry flowers (DF), fresh stems (FS) and dry stems (DS). The GC-MS analyses of the oils revealed the presence of forty two compounds for FF, thirty seven compounds for DF, forty eight compounds for FS and thirty six components for DS. The total yields of the volatile essential oils were respectively: 97.9% (FF), 88.6% (DF), 96.4% (FS) and 87.4% (DS) with the prevalence of α-pinene (FF 32%, DF 16.1%, FS 11.5%, DS 17.4%), β-pinene (FS 8.1%, DS 8.9%), α-phellandrene (FF 8.5%, DF 24.3%), fenchylacetate (FF 10.4%, FS 7.3%, DS 8.8%), elixene (DF 6.3%, FS 5.4%), aristolene (FF 5.4%, FS 7.2%, DS 6.8%), caryophyllene oxide (FS 7.6%) and carotol (FF 13.9%, DF 10.7% FS 18.8%, DS 10.8%). The essential oils were tested againest 9 bacteria and showed a good antibacterial activity against almost food-borne pathogens and clinical isolated microorganisms at a concentration of 128 µg/mL Minimum inhibitory concentration (MIC) values for all the bacteria were ranged between16 µg/mL and 80 µg/mL.

Keywords: *Ferula vesceritensis*; Apiaceae; essential oil; hydrodistillation; antibacterial activity.

1. Introduction

Essential oils and volatile constituents extracted from Aromatic plants are frequently used in folk medicine for prevention and treatment of different human diseases, such as bacterial and viral infections [1], as well as to prevent the growth of food borne bacteria specially Enterotoxins produced by *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* which are responsible for toxicity of the intestinal tract causing nausea and diarrhea [2].

The genus *Ferula* (Apiaceae) is represented by more than 170 species distributed in Central Asia and the Mediterranean region [3]. The Algerian flora comprises 5 species of *Ferula* from which 2 are endemic [4,5], including the presently reported species *F. vesceritensis* Coss. & Dur. [6-9].

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Monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes characterized the compositions of most reported *Ferula* essential oils which exhibited antimicrobial, antioxidant, antiepileptic, anticancer and ferulenol toxicity activities [9-24]. In continuation of our works on Apiaceae essential oils [25-28], we report here the GC and GC-MS analyses and the antibacterial activity of four essential oils of fresh and dry flowers and stems of the endemic species *Ferula vesceritensis* Coss. & Dur. "kelkha", traditionally used to treat fever and headache [8].

2. Materials and Methods

2.1. Plant materials

Fresh aerial parts of *Ferula vesceritensis* Coss. & Dur [4,5] were collected from Ghardaia (Septentrional Algerian Sahara) in March 2011. The plant was authenticated by Pr. Gérard De Bélair, a voucher specimen was deposited at the herbarium of the Laboratory of Therapeutic Substances, University of Constantine 1, Algeria (LOST ZK Fv03/11).

2.2 Isolation of the essential oils

From the freshly collected plant, two parts of separated flowers and stems were dried at room temperature. The fresh flowers and stems (FF, FS) were hydrodistilled, for 3 h, in a Clevenger-type apparatus. Then, the dried flowers and stems (DF, DS) were also likely hydrodistilled, yielding yellow oils of FF (1.8%), DF (1.6%), FS (1.6%), DS (1.4 %) (w/w), which were dried over anhydrous Na2SO4 and conserved in sealed brown vials at 4-6 °C, prior to further analysis.

2.3. Gas chromatography GC-FID

The essential oils were analysed on a Shimadzu gas chromatograph Model GC2010, equipped with a DB-5 MS column (30m x 0.25mm; 0.25µm), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Hydrogen was used as carrier gas (1.0 mL/min); injection in split mode (1:60); injector and detector temperatures were 250°C and 280°C respectively. The essential oil was diluted in hexane (1/30) for the analyses.

2.4. Gas chromatography-mass spectrometry GC-MS analyses

The essential oils were analysed on a Shimadzu gas chromatograph Model GC2010 coupled to a Shimadzu MS model QP2010, equipped with a DB5 MS column (30m X 0.25mm; 0.25µm), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Helium was used as carrier gas (1.0 mL/min): injection in split mode (1:30); injector and detector temperatures were 250°C and 280°C respectively. The MS working in electron impact mode at 70 eV; electron multiplier, 2500 V; ion source Temperature, 180°C; mass spectra data were acquired in the scan mode in *m/z* range 33-450.

2.5. Identification of components

Retention indices of all the components were determined by Kovats method. The compounds assayed by GC were identified by comparing their retention indices with those of reference compounds in the literature and confirmed by comparison of their mass spectra with those of reference substances for major components of the oils [29-33].

2.6. Antibacterial activity testing

The antibacterial activity of the essential oils was evaluated against the tested organisms according to Clinical and Laboratory Standards Institute [34]. Freshly cultured bacterial suspensions in Mueller Hinton Broth were standardized to a cell density of $1.5x10^8$/mL (McFarland No. 0.5). The essential oils of *F. vesceritensis* were tested against nine of microorganisms (food spoilage and food-borne pathogens and clinical strains).

The bacterial pathogens including food spoilage bacteria *Pseudomonas aeruginosa* ATCC 27853, and food-borne pathogens namely, *Enterobacter aerogenes, Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 43300, the reference strains were obtained from the Pasteur Institute (Algiers) while *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella*

pneumoniae, and *Morganella morganii* which were clinical isolated from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods (clinical isolation) [34].

Susceptibility of the bacterial strains to the essential oils was investigated using the disk diffusion method. Empty sterilized discs were impregnated by essential oils which were diluted with 20 mL of DMSO used for antibacterial activity assays. The same volume (20 mL) of DMSO was used as control. The diameters of inhibition zones were measured and compared with those suggested by Clinical and Laboratory Standards Institute [34], (sensitive P≥15 mm). Ampicillin (10 µg/disc) was used as a positive reference standard to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 37 ºC for 24 h. Antimicrobial activity was evaluated by measuring the inhibition zone (IZ) in mm against the tested strains.

The experiments were repeated in triplicate and the results were expressed as average values.

2.7. Determination of minimum inhibitory concentration (MIC)

All the nine tested bacteria were susceptible to the essential oils, hence the minimum inhibitory concentrations of the essential oils were determined using the agar dilution method and different concentrations of the essential oils were included in Mueller-Hinton agar plates. The minimum inhibitory concentration (MIC) was defined as the concentration at which no colony was observed after incubation [34].

3. Results and Discussion

3.1. Chemical composition of the essential oils

The essential oils of FF, DF, FS, DS were obtained by hydrodistillation, The GC and GC–MS analyses of the oils revealed the presence of forty-four compounds for FF, fifty compounds for DF, thirty-nine compounds for FS and thirty-seven compounds for DS. Comparative study showed that the amount of total volatiles of essential oils were 97.9% (FF), 88.6% (DF), 96.4% (FS) and 87.4% (DS), respectively (Table 1), the highest value of essential oil was obtained for the fresh parts of the plant.

The main components of the essential oils were α-pinene (FF 32%, DF 16.1%, FS 11.5%, DS 17.4%), β-pinene (FS 8.1%, DS 8.9%), α-phellandrene (FF 8.5%, DF 24.3%), fenchylacetate (FF 10.4%, FS 7.3%, DS 8.8%), elixene (DF 6.3%, FS 5.4%), aristolene (FF 5.4%, FS 7.2%, DS 6.8%), caryophyllene oxide (FS 7.6%) and carotol (FF 13.9%, DF 10.7% FS 18.8%, DS 10.8%).The essential oils analysed in this research showed important differences in their quality and quantity of the components, which are totally different from the essential oil of the leaves of the Algerian species *F. vesceritensis* collected in the South Eastern Algerian (May 2010), which was mainly characterized by 9, 9-tetradecadiyne (24.72%), germacrene -D (24.51%), farnesene (8.57%) and α-bisabolene (8.57%) [9]. This may be due to the difference of growth stage and locality and time of collection.

It's important to mention that's the first time for the genus *Ferula* that, α-phellandrene, fenchyl acetate, elixene, aristolene and carotol are found as main components. In agreement with the literature, the present oils are constituted with high levels of bicyclic monoterpenes (α-pinene and β-pinene). α-Pinene together with β-pinene have been found as major components in essential oils of *F. gummosa* Boiss. fruits (18.3%, 50.1%) [17]. *F. szovitsiana* D.C (8.0%, 6.7%)[13], *F. badrakema* (10.9%, 45.8 %) [15], *F. communis* L. subsp. *glauca* growing in Marche (Central Italy) (0.3-24.2%, 0.1-14.7%). [19], *F. lycia* Boiss. (59.9%, 19.0%) [35], *F. gummosa* Boiss. from Kashan, Iran (58.8%, 5.7%) [37], *F. jaesekheana* Vatke (30%, 16.2%) [13], *F. stenocarpa* Boiss. & Hausskn (48.8%, 30.1%) [38] and *F. assafoetida* (21.30 %, 47.10%) [40], respectively. Even though, α-pinene, without the main presence of β-pinene, was detected in the essential oils of *F. foliosa F. lutea*, *F. communis* from Algeria, *F. flabelliloba* and *F. ovina* (35.10% , 25.8% , 20.9%, 10.0%, 8.2%, respectively) [14, 36, 39, 41, 10]. It’s noteworthy that caryophyllene oxide (13.90%) was also mainly present in the essential of *Ferula communis* L. subsp. *glauca* growing in Marche Central Italy [19].

Table 1. Chemical composition of *F. vesceritensis* Coss. & Dur. oils from FF, DF, FS, and DS.

Compound ID	RIE[a]	RIL[b]	FF %	DF %	FS %	DS %
Heptane	700	700	0.3	-	-	-
2-Methylheptane	765	766	-	-	-	0.9
Isovaleric acid	836	827	0.2	-	0.2	-
n-Hexanol	871	863	0.1	-	-	-
2-Ethoxyethylacetate	904	903	0.1	-	-	-
α-Thujene	932	924	0.4	1.1	0.5	-
α-Pinene	940	932	32.0	16.1	11.5	17.4
Camphene	954	946	0.7	0.3	0.6	1.0
β-Pinene	979	974	-	1.9	8.1	8.9
Myrcene	991	988	0.2	1.1	0.2	-
δ-2-Carene	998	1001	-	0.9	-	-
δ-3-Carene	1007	1008	0.1	0.4	-	-
α-Phellandrene	1005	1002	8.5	24.3	-	-
3-Ethyl-4-methylpentanol	1022	1020	-	-	0.1	-
p-Cymene	1026	1020	-	1.8	0.1	-
Limonene	1029	1024	0.5	3.4	0.7	-
β-Phellandrene	1030	1025	-	2.7	-	-
E- β -Ocimene	1046	1044	-	2.4	-	-
γ -Terpinene	1062	1054	-	1.8	0.4	-
cis-sabinene hydrate	1070	1065	0.6	-	-	-
Fenchone	1087	1083	0.6	-	0.2	0.8
Linalool	1097	1095	0.4	-	0.3	-
α-pinene oxide	1098	1099	0.5	-	0.3	-
Isophorone	1121	1118	-	-	-	1.3
trans-p-Mentha-2,8-dienol	1125	1119	0.3	-	-	-
α-Campholenal	1126	1122	-	-	0.2	0.2
cis-p-Mentha-2,8-dien-1-ol	1138	1133	-	-	-	0.7
trans-Pinocarveol	1139	1135	0.8	-	0.6	2.9
cis-Verbenol	1140	1137	2.5	-	1.8	3.5
Camphor	1146	1141	-	-	-	0.3
p-menthone	1153	1148	-	0.6	-	-
Pinocarvone	1165	1160	0.5	-	0.3	0.6
Terpinen-4-ol	1178	1174	0.3	0.2	0.2	-
α-Terpineol	1192	1186	0.1	0.2	0.2	-
Myrtenol	1194	1194	0.6	-	0.6	-
Myrtenal	1196	1195	0.2	-	0.2	2.9
nor-Davanone	1227	1228	-	0.3	-	-
Fenchyl acetate	1232	1229	10.4	0.9	7.3	8.8
Bornyl acetate	1285	1283	1.1	0.2	0.8	1.1
p-Cymen-7-ol	1290	1289	0.2	-	0.2	0.3
2-Undecanone	1294	1293	-	-	0.1	0.4
α-Cubebene	1350	1345	0.1	-	0.1	0.9
α -Copaene	1374	1377	0.2	0.2	0.4	0.4
Geranyl acetate	1380	1379	-	-	-	0.3
β-Cubebene	1388	1387	-	-	0.2	0.3
3-Dodecanone	1389	1389	0.2	-	-	0.3
β-Elemene	1391	1389	0.3	2.8	0.7	-
α-Funebrene	1402	1402	-	-	0.3	-
β-caryophyllene	1419	1417	0.2	0.3	2.3	-
β-Gurjunene	1433	1431	0.9	0.5	1.4	2.4
Aristolene	1444	1444	5.4	0.3	7.2	6.8
α-Acoradiene	1464	1460	0.2	-	-	-
γ-Gurjunene	1477	1475	-	-	0.2	-
γ-Muurolene	1480	1478	2.8	0.4	3.9	0.4
α-Muurolene	1495	1500	0.7	-	0.9	0.6
Elixene	1504	1505	-	6.3	5.4	-
trans-Cycloisolongifol-5-ol	1505	1513	-	-	0.2	-
Germacrene A	1506	1508	-	0.2	0.5	0.4
γ-Cadinene	1510	1514	2.7	0.8	2.0	1.0
α-Copaen-11-ol	1534	1539	0.5	-	1.1	0.5
Caryophyllenyl alcohol	1570	1570	-	0.1	0.4	1.1
Germacrene D-4-ol	1575	1574	-	0.4	4.0	-
Spathulenol	1578	1577	-	0.7	0.4	1.5
Caryophyllene oxide	1583	1582	4.3	2.3	7.6	4.6
Globulol	1585	1590	-	-	-	1.5

Compound ID	RIE[a]	RIL[b]	FF %	DF %	FS %	DS %
Viridiflorol	1593	1592	-	-	0.2	0.1
Carotol	1595	1594	13.9	10.7	18.8	10.8
Guaiol	1598	1600	-	0.3	-	-
Alloaromadendrene epoxide	1641	1639	1.1	-	-	-
Cubenol	1646	1645	-	-	-	1.5
α-Cadinol	1654	1652	2.2	0.5	2.5	
α-Selina-11-en-4-ol	1660	1658	-	0.5	-	-
Total (%)			**97.9**	**88.6**	**96.4**	**87.4**

FF: fresh flowers, DF: dry flowers, FS: fresh stems, DS: dry stems.
RIE[a] Retention index calculated from relative retention times; RIL[b] Literature Retention index [29-33].
2-Methylheptane [30], 3-Ethyl-4-methylpentanol [31], Aristolene [32], Elixene [33].

According to the literature, α-pinene and β-pinene are mainly found in the present essential oils confirming that these monoterpenes are chemotypes of *Ferula* essential oils. In contrast, variations in the compositions of essential oils isolated from fresh flowers and dry flowers on the one hand and from fresh stems and dry stems on the other hand may be explained on account of some important facts biotransformation of monoterpenes [42]. The volatility of monoterpenes causes problems during biotransformation, such as losses of both substrates and products. Biotransformation of terpenes often proceed along several metabolic pathways leading to a mixture of products. Monoterpenes are relatively unstable compounds. They can, for example, undergo spontaneous autoxidation.

3.2. Antibacterial activity

From the GC and GC-MS results it appears that the present *F. vesceritensis* essential oils are rich with α-pinene and β-pinene which have been already reported to possess strong antimicrobial activities [43]. These monoterpenes exert their antimicrobial activity on microorganisms through the disruption of bacteria membrane integrity [44]. The hydrophilic cell wall structure of Gram-negative bacteria, constituted essentially by a lipo-polysaccharide, blocks the penetration of hydrophobic components of oils and for this reason, Gram-positive bacteria are found to be more sensitive to the essential oils effect [45].

Thus, we've been interested to evaluate the antibacterial activity of the present essential oils by the use of the disc diffusion method and MIC values (Tables 2 and 3). The tested essential oils inhibited strongly the growth of all bacterial strains at MIC values ranging between 16 µg/mL and 80 µg/mL. Dry flowers and stems essential oils showed the best antibacterial activity comparing with fresh flowers and stems essential oils because of the high percentages of α-pinene together with β-pinene as main components (Table 3). The best activity was exhibited against *Staphylococcus aureus ATCC 43300, Pseudomonas aerugina ATCC 27853, Escherichia coli ATCC 25922, Morganella morganii, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli* strains with 30 mm, 29 mm, 27 mm, 25 mm, 24 mm, 24 mm and 24 mm inhibition zone diameters, respectively (Table 2).

Unlike the previous report on the essential oil of leaves of *F. vesceritensis* [9], the important key of the current study is that it includes an investigation on fresh and dry flowers and stems essential oils and their influence on chemical composition and also on the biological activity of this plant. In the present work, a relationship between the composition of the *Ferula* essential oils studied and antibacterial activity was observed because of the richness with α-pinene and β-pinene (markers of antibacterial effect).

In addition, it's the first time in *Ferula* oil that α-phellandrene, fenchylacetate, elixene, aristolene and cartol are found as major components. The antibacterial activity of these oils was tested by the use of the disc diffusion method against nine microorganism namely *Escherichia coli* ATCC 25922, *Escherichia coli*, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus*, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Morganella morganii*. The best antibacterial activity was shown against six strains, *Staphylococcus aureus ATCC 43300, Pseudomonas aerugina ATCC 27853, Escherichia coli ATCC 25922, Morganella morganii, Staphylococcus aureus and Escherichia coli.* So we can conclude that the studied oils have a strong and broad spectrum antibacterial effect against bacterial strains. For this reason, the use of the essential oils of *F. vesceritensis* may be very important for pharmaceutical and cosmetic industries which can substitute synthetic additive (hydrophobic chemical addition) in the

food industry, where they can be considered as natural preservatives and can be used as an herbal medicine against food spoilage microorganisms.

Table 2. Antibacterial activity of different essential oils of *F. vesceritensis* Coss. & Dur. (inhibition zones, mm).

Microorganism	Inhibition zone (mm)					
	Antibiotics (10 µg /mL)		Essential oils (128 µg /mL)			
	Ampicillin[a]	Gentamicin[a]	FF	DF	FS	DS
Escherichia coli ATCC 25922[b]	18	22	25	27	28	24
Escherichia coli[c]	-	-	21	24	22	29
Staphylococcus aureus ATCC 43300[b]	30	-	27	30	25	25
Staphylococcus aureus[c]	-	13	24	23	23	22
Pseudomonas aerugina ATCC 27853[b]	-	-	18	23	27	30
Pseudomonas aerugina[c]	-	12	18	20	22	26
Klebsiella pneumoniae[c]	14	21	24	20	14	16
Morganella morganii[c]	-	-	20	25	15	10
Enterobacter aerogenes[c]	-	-	23	20	12.5	10

FF: fresh flowers, DF: dry flowers, FS: fresh stems, DS: dry stems; [a] Control; [b] Obtained from the Pasteur Institute (Algiers); [c] Clinical isolates from the laboratory of bacteriology (CHU Constantine, Algeria).

Table 3. Comparison of the MIC values (µg/mL) of different essential oils of *F. vesceritensis* Coss. & Dur.

Microorganism	MIC[a] (µg /mL)					
	Antibiotics (µg /mL)		Essential oils			
	Ampicillin[b]	Gentamicin[b]	FF	DF	FS	DS
Escherichia coli ATCC 25922[c]	10	10	40	80	40	40
Escherichia coli[d]	-	-	40	80	40	40
Staphylococcus aureus ATCC 43300[c]	5	-	80	16	40	16
Staphylococcus aureus[d]	-	15	80	16	40	16
Pseudomonas aerugina ATCC 27853[c]	-	-	40	80	40	40
Pseudomonas aerugina[d]	-	5	40	80	40	40
Klebsiella pneumoniae[d]	10	5	32	80	16	40
Morganella morganii[d]	-	-	40	80	40	40
Enterobacter aerogenes[d]	-	-	40	80	16	16

FF: fresh flowers, DF: dry flowers, FS: fresh stems, DS: dry stems, [a]MIC: minimum inhibitory concentration; [b]Control ; [c]Obtained from the Pasteur Institute (Algiers); [d]Clinical isolates from the laboratory of bacteriology (CHU Constantine, Algeria)

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