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ORIGINAL PAPER

Algerian wild fennel essential oils: chromatographic profile, acute toxicity, antioxidant, and antimicrobial activities

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Abstract

The present study deals with the characterization of essential oils from umbels and seeds of Algerian wild (bitter) fennel (*Foeniculum vulgare* Mill. Var *vulgare*) by determining the chromatographic profile, lethal dose (LD50), antioxidant and antimicrobial activities, as well as a kinetic modeling study of the extraction of the seed-based essential oils. The extraction of essential oils (EOs) was performed by hydrodistillation using Clevenger for 3.5 and 6 h for the umbels and seeds, respectively. The two mathematical models from the experimental data show a good fit with an R^2 of 99.99 and 98.94%. GC/MS analyses of fennel EOs showed that fennel was rich in different oxygenated monoterpenes compounds. However, while fenchone was the main compound in fennel seeds (FSEO), fennel umbel EO (FUEO) mainly contained α-pinene, o-cymene, sylvestrene, fenchone, Endo-fenchyl acetate, and carvacrol. The acute toxicity study of FSEO showed a lethal dose (LD50) of 4.9085 ± 0.1213 g/kg body weight in mice. Based on the free radical scavenging method using BHT as a positive control, the IC50 values were 9.9658 ± 0.057 mg/mL and 0.4570 ± 0.0456 mg/mL for FSEO and BHT, respectively. The study of antimicrobial activity in two gram-negative bacteria: *Echerichi coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), and one gram-positive bacterium: *Bacilus subtilis* (ATCC 6633), as well as two fungal strains: *Candida albicans* (ATCC 10,231), *Saccaromyces cerevisiaes* (ATCC 9763), revealed that the fungal strains were more susceptible to FSEO and showed a significant fungicidal effect. The results of this study highlight the high quality of Algerian wild fennel, and the possibility of recovering it for use in the pharmaceutical, cosmetic, and food industries.

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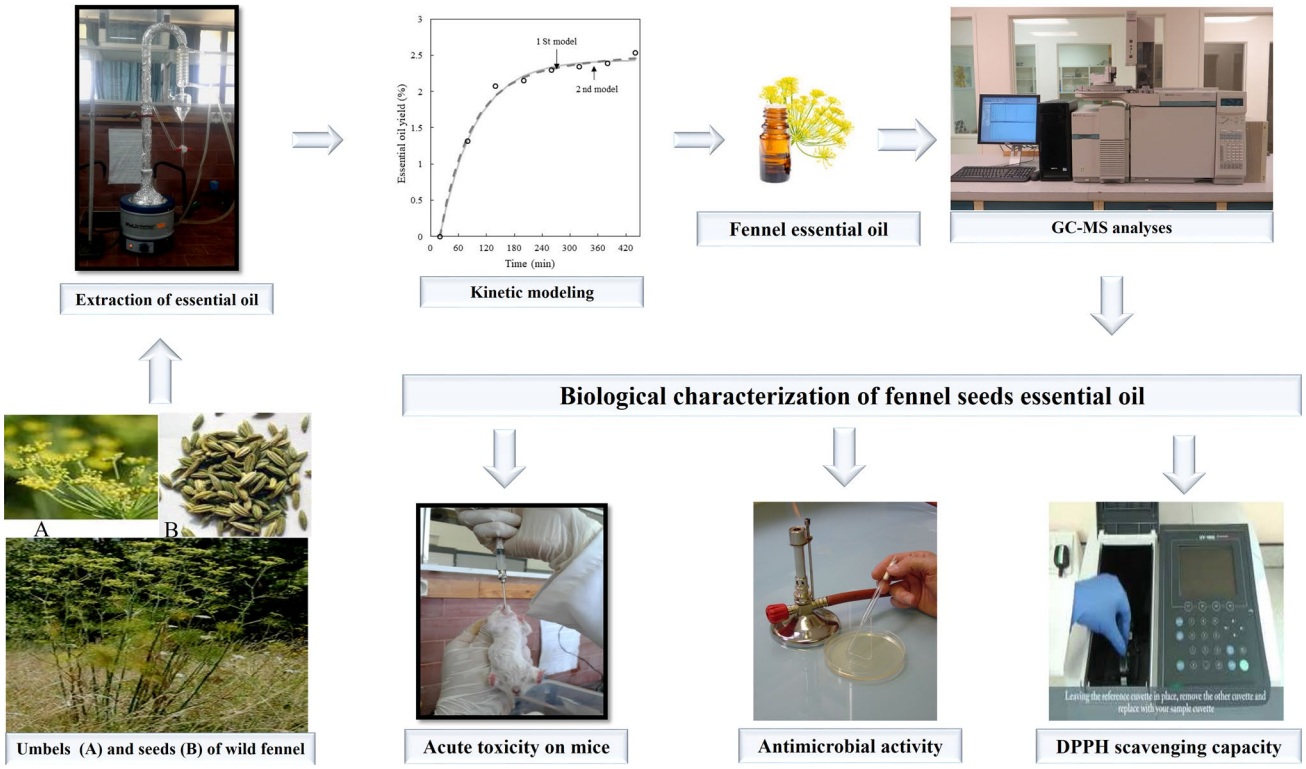
Graphical abstract



Keywords Essential oils (EO) · *Foeniculum vulgare* Mill. Var *Vulgare* · Antimicrobial activity · Acute toxicity · Extraction kinetics

Introduction

Traditional medicine has used natural-based materials to treat various diseases. Recently, medicinal and aromatic plants (MAPs) have become a vital natural resource of medication not only in developing countries but all over the world (Yahaya Gavamukulya et al. 2014). Thus, the World Health Organization estimates that more than 80% of the world population uses traditional medicine to treat diseases, illnesses, and other health problems (Alabri et al. 2013; Alhakmani et al. 2013). MAPs have been the subject of essential studies on antimicrobials and natural antioxidants (Singh et al. 2006). It is worth noting that about 20% of plants known and studied for their pharmaceutical characteristics have improved the treatment of infections and cancers (Altemimi et al. 2017). Currently, scientists are placing great interest in natural plant-derived substances to replace synthetic antioxidants applied in industry. Indeed, numerous studies have proved that such synthetic compounds have side effects and become more dangerous for human health (Ahmed et al. 2019). Similarly, the resistance developed by pathogenic microorganisms to synthetic antibiotics leads to the exploitation and valorization of these natural gifted substances (Roby et al. 2012; Kwiatkowski et al. 2017), including the essential oils (EO) and non-volatile substances.

Wild fennel (*Foeniculum vulgare* Mill, Var *Vulgare*) is among the spontaneous species frequently applied in traditional medicine. Local population knows it as "BESBES LAKHLA or EL VESVES IYEGHZER" and widely uses it to treat digestive disorders such as dyspepsia, bloating, intestinal pain, and as a vasodilator. It is a biennial MAP belonging to the Apiaceae (*Umbelliferae*) family (Purkayastha et al. 2012), native to the shores of the Mediterranean (Burkhardt et al., 2015; Gheisari Zardak et al. 2016). Various countries have developed its culture in different areas of the world (Bedini et al. 2016), to widely use it in the food, pharmaceutical, perfumery, and cosmetics industries (Zuobing et al. 2017). Thus, fennel has a high commercial value because of its broad potential for therapeutic applications such as balsamic, cardiotonic, digestive, lactagogue, tonic, antispasmodic, anti-inflammatory, expectorant, diuretic and laxative (Damjanovic´, 2004; Mazandrani et al. 2015). Fennel can take part in the treatment of respiratory problems (Solana et al. 2014; Pacifico et al. 2015; Pavela et al. 2015), kidney

stones (Gholami Zali et al. 2017; Rahimmalek et al. 2014), nervous disorders (Pavela et al. 2015; Hatami et al. 2017), menstrual disorders (Ostad et al. 2004, Pavela et al. 2015), menopausal disorders, hirsutism, osteoporosis (Hassanpour et al. 2017), and also has carminative, analgesic (Hatami et al 2017), antibacterial, antifungal, antithrombotic, antioxidant, estrogenic, antidiabetic, gastroprotective, hepatoprotective (Telci et al. 2009; Rather et al., 2012; Pavela et al., 2015; Gholami Zali et al. 2017), antitumor and cytoprotective properties (Majdoub et al. 2017).

The third European pharmacopeia has reported two fennel drugs based on bitter (*Foeniculum Vulgare* Miller *spp. Vulgre* var *Vulgare*) and sweet (*Foeniculum vulgare* Miller subsp. *vulgare* var. dulce (Miller) Thellung) fennel seeds (Damjanovic et al. 2004). Fennel is also considered a flavor used in the food, beverage, and cosmetics industry (Salama et al. 2013; Baby et al. 2016; Benmoussa et al. 2016; Zuobing et al. 2017).

Several studies reported that fennel EO shows chemical polymorphisms significantly different depending on variety (Diäaz-Maroto et al. 2005). Some authors have reported that *trans* anethole (Rahimmalek et al. 2014), fenchone (present in bitter varieties), (Damjanovic et al. et al. 2005; Hatami et al. 2017), estragol (methyl chavicol) (Diäaz-Maroto et al. 2005; M. Krizman et al., 2006; M., Hammouda et al., 2013), limonene (Akgul et al. 1988; Bahmani et al. 2015), and α-pinene are the main EO compounds (Senatore et al. 2013; Baby et al. 2016; Zuobing et al. 2017). Muckensturm et al. (1997), Gross et al. (2009), and Telci et al. (2009) recognized three chemotypes: the estragole type, the estragol/anethole type, and the anethole type, while Senatore et al. (2013) cited two chemotypes (*trans*-anethole and estragole) of bitter fennel.

In Algeria, phytochemical studies on local fennel EO are scarce. We cite those published by Zoubiri et al. (2010, 2011), Ouis et al. (2014) on the EO of cultivated fennel, and that of Lazouni et al. (2007) on wild fennel. This latter will be the object of the present study. In this context, we will present the chemical composition of their EO obtained from seeds and umbels (obtained after post-harvesting treatment). In the same way, we will illustrate a study on the toxicity of FSEO and their antioxidant and antimicrobial potential.

Materials and methods

Plant material

Identifying this plant at the National School of Agronomy (ENSA-Algiers) confirmed a wild fennel species. The wild fennel umbels (with mature green seeds) used in this study were harvested in November 2015 in the locality of Blida (Algeria), located 45 km southwest of Algiers. This plant material shade-dried is processed to separate the seeds from the umbels and then stored in paper bags until use.

Water content

The assessment of the water content of the samples involved the use of 3 g of each grain sample to place in an oven at 105 °C under atmospheric pressure for 24 h (Bouallegue et al., 2015). The final samples used for the extraction processes had a water content of 11.90 ± 1.09 and 10.44 ± 0.14 g H_2O/ 100 g^{-1} db (dry basis) for seeds and umbels, respectively.

Treatment and extraction

Extraction of essential oils

Extraction of essential oils (EO) from fennel seeds and umbels, used Clevenger type hydrodistillation with about 40 g and 60 g for 3.5 and 6 h as extraction time, respectively. The solid to liquid ratio was 1:10. with the extraction yield expressed as the ratio of the mass of EO obtained to the mass of dry plant material.

Kinetic modeling of fennel seed essential oils

Because of highly agitated dynamic extraction conditions, a negligible external resistance (NER) characterizes the hydrodistillation. The internal diffusion of water within the matrix was the limiting process. Thus, the literature proposes two mathematical kinetic models to simulate solid–liquid extraction (Benyoussef et al. 2013; Segovia et al. 2014). The first equation (Eq. 1) consists of one immersion term, while the second (Eq. 2) consists of two immersion terms for the studied matrix.

$$Y = Y_{\max}\left(1 - e^{-\mathrm{kt}}\right) \tag{1}$$

$$Y = Y_{\max}\left(\left(1 - fe^{-k_1 t}\right) - (1 - f)e^{-k_2 t}\right) \tag{2}$$

where Y is the yield at time t, Y_{max} is the maximum yield obtained at equilibrium ($t = \infty$); k, and k_1 and k_2 are the kinetic constants (min^{-1}), f and (1 − f) are the fractions of solute diffusing at two different rates. The second model (Eq. 2) derives from the first model (Eq. 1) by assuming that the solid matrix has two extraction stages; surface interaction and internal diffusion or two types of cell structures; broken and intact. It is worth noticing that this part of the study did not imply umbels because of their poverty in EOs.

Chromatographic analyses of fennel essentials oils

Coupled Gas Chromatography / Mass Spectrometry (GC—MS) allows analyzing EO. In the present case, the mass detector was Hewlett Packard type (model 5973), the HP-5SM column (30 m * 0.25 mm * 0.25 µm) with a stationary phase at 5% phenyl and 95% dimethylpolysiloxane.

The injector temperatures, the GC–MS interface, and the ion source were maintained at 250 °C, 280 °C, and 230 °C, sequentially. The oven temperature programmed for the analysis was 60 °C (8 min), 60–250 °C (2 °C/min), and held at 250 °C for 15 min. The volume injected was 0.2 µL in split mode (1/20), and the carrier gas was helium (purity N60) at 0.5 µL/min. This system allowed identifying the compounds by comparing the retention indices calculated using the homologous series of C8-C28 n-alkanes, and their mass spectrum with those in the literature (Adams 2007) and NIST 02.L (National Institute of Standards and Technology) and Wiley 7n.1 libraries mass spectral libraries.

Biological investigation of fennel essentials oils

These different parts are related only to FSEO. Indeed, FUEO had such a low EO yield that the involving tests were only the yield and the GC–MS analyses without exploiting the kinetic and biological study part.

Acute toxicity study of fennel seeds essential oil

To estimate the lethal dose (LD50) of FSEO, a population of 30 NMR-I mice (25–30 g) was divided into 5 groups (6 mice each). All animals underwent a 12-h fast before the experiment. The first group served as vehicle control and received Twen80 (1%), and the other groups received a single oral dose of FSEO (3, 4, 5, and 6 g/kg) suspended in the vehicle (Turner 1965). Animal mortality and symptoms of toxicity were observed during the first 24 h and the next 22 days. After this period, the dead animals were counted to assess the LD50 using the method described by Miller and Tainter (Randhawa et al. 2009).

Free radical scavenging capacity of fennel seeds essential oil

The study of the antioxidant activity of FSEO was carried out using the free radical scavenging method DPPH (2, 2-diphenyl-1-picrylhydrazyl), and by adopting the protocol described in the literature (Kontogiorgis et al. 2016; Khaled Khodja et al. 2018). BHT (butylated hydroxytoluene) was used as a positive control. 3 ml of FSEO solution (1, 0.8, 0.6, 0.4 and 0.2 mg/mL) and that of BHT (1, 0.8, 0.6, 0.4 and 0.2 mg/mL) were added separately to 1 ml of DPPH solution (0.1 mM) prepared in ethanol. The mixture was shaken in the dark for 30 min, and then the absorbance (Abs) of each sample was measured at 517 nm against pure ethanol using a UV-1800 type SHIMADZO-UV spectrophotometer. The percentage of inhibition of the free radical DPPH was evaluated using the formula of percent inhibition:

$$\text{Inhibition} = [(\text{Abs}_{\text{blank}} \ \text{Abs}_{\text{sample}})/\text{Abs}_{\text{blank}}] \times 100 \quad (3)$$

where $\text{Abs}_{\text{blank}}$ is the absorbance of the control (containing all reagents except samples), and $\text{Abs}_{\text{sample}}$ is the absorbance of the sample incubated after 30 min. From the graph of the radical scavenging activity versus the concentration of the sample, it was possible to determine the sample concentration giving 50% of DPPH scavenging activity (IC50). These tests performed in triplicate allow giving IC50 value as the mean ± standard deviation (SD).

Antimicrobial study of fennel seeds essential oil

Microorganism tests The antimicrobial activity of FSEO carried out at Sandal Research and Development Center in Algiers (Algeria) implied five microbial strains of the ATCC (American Type Culture Collection) type (Table 1).

Qualitative study of antimicrobial activity The qualitative assessment of the antimicrobial activity of FSEO followed the disk diffusion method (Abdelli et al. 2016). It consists of detecting the sensitivity of microbial strains by direct contact with FSEO on an agar culture medium (Muller Hinton for bacteria and Sabouraud for fungi) inoculated by a microbial suspension. Sterile disks (6 mm) soaked with FSEO (15, and 20 µL) put on the surface, underwent an incubation at 37 °C for 24 h for bacteria and 48 h at 27 °C for fungi. The results exclusively come from measuring the diameter of the inhibition halos in mm. FSEO activity implied 4 levels of non-sensitive (0) for a diameter of less than 8 mm, moderately sensitive (+) for a diameter of 8 to 14 mm, sensitive (++) for a diameter of 14 to 20 mm, and very sen-

Table 1 Microbial strains used in the antimicrobial activity of Algerian wild fennel seeds essential oil

N°	Microbial strains	ATCC
Gram-negative bacteria		
01	*Echerichi coli*	8739
02	*Pseudomonas aeruginosa*	9027
Gram-positive bacteria		
03	*Bacilus subtilis*	6633
Fungal strains		
04	*Candida albicans*	10,231
05	*Saccaromyces cerevisiaes*	9763

ATCC American Type Culture Collection

sitive (+++) for a diameter greater than 20 mm (Sfeir et al., 2013). Each strain includes two repetitions.

Quantitative study of antimicrobial activity This part was performed only for yeasts, as they showed significant activity compared to bacteria strains, using the method cited by Hammer et al. (1999). A series of dilutions was prepared from the FSEO from 2 up to 0.03% in the appropriate media previously added with Tween 80 (to solubilize the FSEO in the culture medium) at a final concentration of 1% (Kwiatkowski et al. 2017). The sterile disks were placed aseptically in the dishes and soaked by the microbial suspension. After an adequate incubation, the concentration with no visible microbial growth reveals the minimum inhibitory concentration (MIC).

The disks corresponding to the concentrations ascending from the MIC were removed aseptically from the dishes and placed in the Petri dishes containing the appropriate agar to incubate for 48 h at 27 °C. The minimum fungicidal concentration (MFC) was the concentration with no visible microbial growth.

The MFC/MIC ratio allows the classification type of antimicrobial agent (FSEO): fungicidal (MFC/MIC less than 4) or bacteriostatic (greater than 4) (Sbayou et al. 2014; Bertella et al., 2018).

Statistical analyses

All results included means ± standard deviation (SD), except the LD50 value, which gives the geometric means plus their respective confidence limits (95%). ANOVA analyses define the significant differences ($P < 0.05\%$), then multiple comparison tests of Tukey's post hoc test were applied between the averages. The statistical analyses have used Software XLStat (XLStat, Paris, France).

Results and discussion

Kinetic modeling of fennel seeds essential oil

The FSEOs' kinetic presented in Fig. 1 notes three essential steps for the extraction process after heating the plant material for about 20 min. The first one with about 52% of FSEO was linear rapid (60 min). The second was hyperbolic slow (60 min to 360 min), including recovering about 94% of FSEO. The equilibrium stage corresponded to the last and final step (after 360 min). Subsequently, the chromatographic and biological measurements fixed this FSEO extraction time of 6 h.

The literature reported that the kinetics of FSEO extraction gives three specific steps: firstly, a linear increase in yield presenting extraction of surficial EO, then a slow evolution in yield, and finally, the horizontal line corresponding to the end of extraction (Benmoussa et al. 2016).

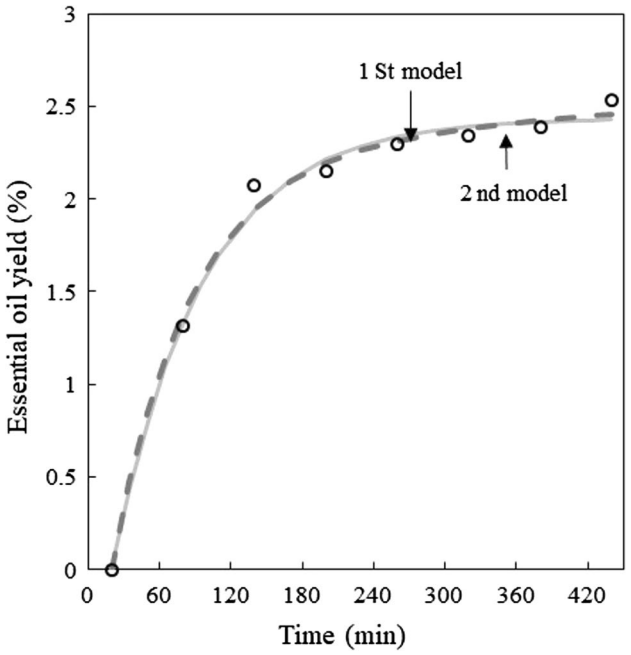


Fig. 1 Kinetics of essential oil extraction of wild fennel seeds (points: experimental data; lines: model)

Table 2 Parameters of models fitted wild fennel seeds essential oil kinetic extraction

Model type	Unique site	Two-sites		
Parameter	k (min)	f	k_1 (min^{-1})	k_2 (min^{-1})
Value of parameter	0.013	0.807	0.016	0.003
Adjusted R^2 (%)	99.99	98.94		

As shown in Fig. 1 and Table 2, the two mathematical models reasonably fit the extraction kinetics with significant values of adjusted R^2. For the single-site model, it is worth noting a kinetic constant (k) value of 0.013 min^{-1}, which reveals a rapid extraction till the equilibrium stage. This model explains the presence of one type on the matrix from which the EO diffuses with this constant rate until exhaustion.

For the two-site model, it is worth perceiving a first kinetic constant with $k_1 = 0.016$ min^{-1}, revealing that firstly we assisted quick extraction processing and the main fraction 80.7% ($f = 0.807$) of FSEO was achieved. However, a small value of the second kinetic constant $k_2 = 0.03$ min^{-1} is noted; it reveals that we have a slow extraction of the remaining fraction of FSEO and reached the equilibrium extraction stage. However, it does not conclude that the unique-site model can better explain the kinetic profile of FSEO extraction.

Table 3 Yields of fennel essential oil from different origins and for different plant parts

Origin	Part of plant (Yield)[authors]
Algeria	Umbels (0.293 ± 0.016%)[a]; Seeds (2.176 ± 0.002%)[a]
Algeria	Seeds (1.82–2.15%)[b]
Egypt	Seeds (1.95; 0.98–2.8%)[c]
Morocco	Seeds (2.13–3.67%)[d]
Tunisia	Seeds (1.2–5.06%)[e]
Turkey	Seeds (5.6%); Steams (1.2%); Leaves (1.7%); umbels with flours (2.1%); Flowers (2.2%)[f]; Seeds (3.5–10.3 mL/100 g)[g]
Brazil	Leaves (0.21–0.30%); Seeds (0.9–3.77%)[h]
Iran	Foliage (0.3–1.5%); Seeds (1.2 à 2.78%)[i]
Indonesia	Seeds (2.004%)[j]
India	Seeds (0.83–1.20%)[k]

a: Present study; b: Lazouni et al. 2006 and Lazouni et al. 2007; c: Roby et al. 2012; Hammouda et al. 2013; d: Ghouati et al. 2014; Abdellaoui et al. 2017; Khammassi et al. 2018; f: Akgul et al. 1988; g: Telci et al. 2019; h: Stefanini et al. 2006b; i: Ehsanipour et al. 2011; j: Damayanti et al. 2012; k:Gonzalez-Rivera et al. 2015; Singh et al. 2006

Yield on essential oils from seeds and umbels of Algerian wild fennel

EOs obtained from umbels and seeds had a golden yellow (FUEO) and pale yellow (FSEO) color with a characteristic odor. The EO yield was about 0.293 ± 0.016% and 2.176 ± 0.002% for umbels and seeds, respectively. Stefanini et al. (2006a), Ilić et al. (2019), and Rezaei-Chiyaneh et al. (2019) reported that seeds were the richest organ in EOs. Our results are comparable to those reported in the literature for wild fennel of different origins (Table 3). The significant variability observed (Table 3) on EO yield is related to various factors such as environmental conditions, geographical origin, genetic factors, maturity and farming practice conditions, and extraction process and time (Telci et al. 2009; Bahmani et al. 2015; Benmousa et al. 2016; Abdellaoui et al. 2017; Ahmed et al. 2019).

Chromatographic profile of essential oils from seeds and umbels of Algerian wild fennel

Table 4 gathers the results of the chromatographic (Fig. 2) analyses of the EOs. We noted a significant difference for the EOs of seeds (FSEO) and umbels (FUEO) of Algerian

Table 4 Chemical composition of essential oil from umbels and seeds of Algerian wild fennel

N°	R_t (min)	RI	Chemical formula	Chemical compound	Ps (%)	
					Umbels	Seeds
01	9.135	939	$C_{10}H_{16}$	α-pinene	22.463	1.304
02	9.928	954	$C_{10}H_{16}$	Camphene	0.574	0.356
03	11.450	975	$C_{10}H_{16}$	Sabinene	–	0.216
04	11.593	979	$C_{10}H_{16}$	β-pinene	1.386	0.062
05	12.674	990	$C_{10}H_{16}$	Myrcene	1.994	2.134
06	13.420	1002	$C_{10}H_{16}$	α-phellandrene	3.684	0.640
07	14.870	1026	$C_{10}H_{14}$	o-Cymene	18.309	–
08	15.082	1030	$C_{10}H_{16}$	Sylvestrene	8.869	–
09	15.219	1029	$C_{10}H_{16}$	Limonene	–	8.664
10	17.229	1059	$C_{10}H_{16}$	γ-Terpinene	–	0.881
11	19.215	1086	$C_{10}H_{16}O$	Fenchone	13.018	83.328
12	21.846	1122	$C_{10}H_{18}O$	*Trans*-pinene hydrate	–	0.167
13	23.287	1146	$C_{10}H_{16}O$	Camphor	–	1.578
14	25.793	1177	$C_{10}H_{18}O$	Terpinen-4-ol	–	0.097
14	28.572	1220	$C_{12}H_{20}O_2$	Endo-Fenchyl acetate	13.165	–
15	33.102	1285	$C_{12}H_{20}O_2$	Isobornyl acetate	0.755	–
16	35.445	1299	$C_{10}H_{18}O$	Carvacrol	6.599	–
17	45.575	1485	$C_{15}H_{24}$	Germacrene-D	1.653	–
Total identified					92.158	99.763
Monoterpene hydrocarbon					57.279	14.257
Oxygenated monoterpene					33.226	85.506
Sesquiterpene hydrocarbons					1. 653	–
Refraction index					1.4745	1.4618

N°: is compounds number; R_t: retention time; KI: Retention Index; and Ps: mean percentage

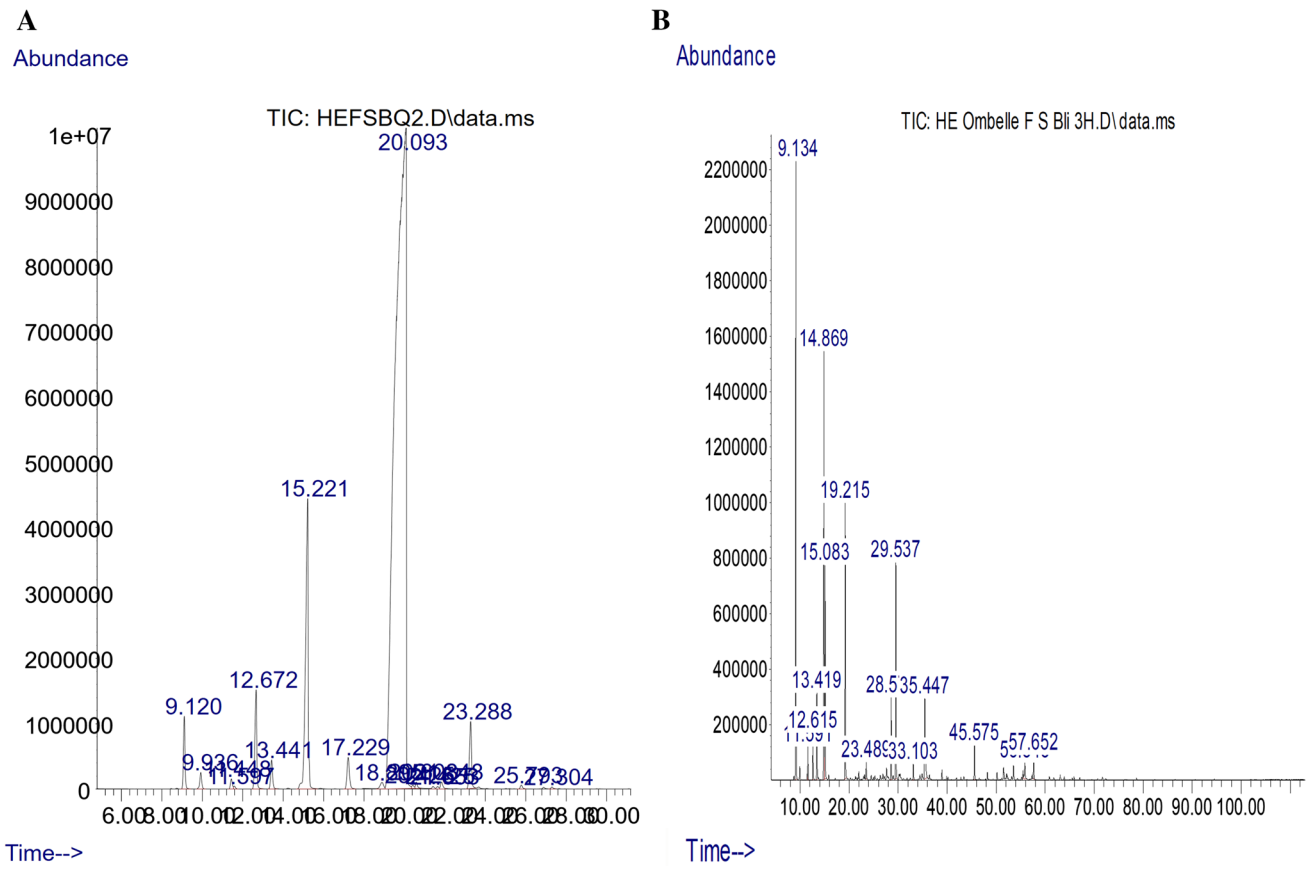


Fig. 2 GC–MS chromatograms of fennel seeds **A)** and umbels **B)** essential oils

wild fennel. Thus, while FSEO has oxygenated monoterpenes (85.476%) with fenchone (83.63%) as the main compounds and monoterpenes with limonene (8.695%) as another important compound, FUEO was rich in monoterpenes (57.755%), mainly α-pinene (23.513%), β-pinene (1.386%), Myrcene (1.994%), α-phellandrene (3.684%), o-cymene (18.309%) and sylvestrene (8.869%), and oxygenated monoterpenes (33.401%) whose; fenchone (13.637%), carvacrol (6.599%) and Endo-fenchyl acetate (13.165%).

Some compounds were present only in FSEO, such as sabinene, γ-terpinene, *trans*-pinenehydrate, and terpinene-4-ol, or only in FUEO like β-pinene, o-cymene, sylvetrene, Endo-fenchyl acetate, isobornyl acetate, carvacrol, and germacrene-D).

The compounds which were present in the two organs of the plant but in variable contents were principally: α-pinene (22.463, 1.307%), β-pinene (1.386, 0.062%), camphene (0.574, 0.357%), myrcene (1.994, 2.14%), α-phellandrene (3.684, 0.64%), and Fenchone (13.018, 83.63%), respectively.

These results agreed with other works which reported that the chemical composition was very variable between the different parts of the same plant (Senatore et al. 2013) and that seeds were richer in fenchone (4.75 to 13.85) compared to other parts of the plant (Akgul et al. 1988).

Despite the significant difference in the chemical composition of FSEO and FUEO, there was not a notable difference in refraction index values (Table 4) which were 1.4745 and 1.4618, respectively. This concords with the literature reported values (1.484 to 1.508) (Garnéro, 1996), but is different from that (1.55) cited for Indonesian FSEO (Damayanti et al. 2012).

Our results were different from those reported for wild fennel from different regions in the world (Table 5), for which the most abundant chemotypes were: *trans*-anethole, estragole, and fenchone. Besides, we noted that the Sylvestrene detected in FUEO was cited as a trace (1.7%) in the EO of Iranian fennel leaves (Shahmokhtar et al. 2017).

Also, it has been reported that the same variety can produce a variable EO chromatographic profile depending on genetic, climatic, geographic, and agronomic conditions (Badgujar et al. 2014; Zuobing et al. 2017; Bahmani et al. 2015; Rezaei-Chiyaneh et al. 2019), maturity stage of seed, soil quality, phytopathological insects, shine, rain, microorganisms (Stefanini et al. 2006b), the hydrodistillation method, the hydro module (Ilić et al. 2019), adaptive

Table 5 Chemical composition of essential oil of wild fennel from different origins

Origin	Part of plant; Abundant chemical compounds[Authors]
Algeria	Umbels [α-pinene (23.513%), β-pinene (1.386%), myrcene (1.994%), α-phellandrene (3.684%), o-cymene (18.309%), and sylvestrene (8.869%), fenchone (13.637%), carvacrol (6.599%), and Endo-fenchyl acetate (13.165%)][a]
Algeria	Seeds [fenchone (83.63%), Limonene (8.695%)][a]
Algeria	Seeds [Fenchone (31.765%), α-phellandrene (17.087%), Δ-3-carene (16.08%), anethole (10.34%), (9.17%) limonene (70%) + β-phellandrene (30%), pycimene (7.70%), α-pinene (5.12%)][b]
Egypte	Seeds [Anethole (55% to 72%) and fenchone (18 -28%)][c]
Spain	Seeds [Anethole, α-pinene, estragole, fenchone, piperitone oxide and limonene][d]
Morocco	Seeds [*Trans*-anethole (53.20%), fenchone (9.55%), limonene (8.14%), and estragole (5.29%)][e]
Italy	Seeds [α-phellandrene/estragole/anethole, α-pinene/limonene/*trans*-anethole, estragole/α-phellandrene, estragole/α-pinene, and α-phellandrene][f]
Tunisia	Seeds [Estragole (66.09–85%), fenchone (5.18–23.09%), and limonene (4.3–10.25%)][g]
Tadjakistan	Aerial parts [*Trans*-anethole (36.8%); α-ethyl-p-methoxy-benzyl alcohol (9.1%); p-anisaldehyde (7.7%); carvone (4.9%); 1-phenyl-penta-2,4-diyne (4.8%) and fenchyl butanoate (4.2%)][h]
Iran	Seeds [*Trans*-anethole, estragole, fenchone, and limonene (13.7%)][i]
Turkey	Seeds [anethole/estragol chemotype][j]
Turkey	Seeds [Estragole et estragol/fenchone with a maximum of fenchone of 22.9%][k]
Brazil	Steams/leaves [Limonene (34.48–46.11%); *Trans*-anethole (46.11–53.95%)]; Seeds [*Trans*-anethole (77.26–78.25%)][l]
Iran	Leaves [*Trans*-anethole (15.1%), Cis-anethole (7.5%), α-pinène (11.4%), D-limonene (5.6%), α-fenchone (4.4%), endo-fenchol (1.9%), Exo-fenchol (2,4%), germacrene D (1.8%), and sylvestrene (1.7%)][m]

a: Present study; b: Lazouni et al. 2007; c: Hammouda et al. 2013; d: Napoli et al. 2010; e: Ghouati et al. 2014; f: Piccaglia et al. 2001; g: Khammassi et al. 2018; h: i: Bahmani et al. 2015; j: Yaldiz et al. 2019; k: Telci et al. 2019; l: Stefanini et al. 2006b; m: Shahmokhtar et al. 2017

plant metabolism (Roby et al. 2012; Ahmed et al. 2019), which were reported for bitter fennel (Stefanini et al. 2006b) and vary according to the species, origin, and season (Bassyouni et al. 2018). On the other hand, we have exploited the same species (from Dellys; a small Mediterranean town in northern Algeria's coastal Boumerdes province at 50 km), which has shown a different chromatographic profile with the dominance of Fenchone (39.05–43.55%) and trans-anethole (34.79–42.86%) (Dahmani 2014). The dominance of fenchone, a typical Fennel EO compound (Napoli et al, 2010), gives excellent value to these EOs because of their importance in flavors, fragrances, and cosmetic and food preparations (Gonzalez-Rivera et al. 2015). Moreover, this composition is highly interesting for the pharmaceutical industry since the therapeutic effects of EOs correlate with their richness in oxygenated compounds (Yaldiz et al. 2019)..

Acute toxicity (LD50) of essential oils of Algerian wild fennel seeds

The therapeutic index of natural plant substances is set by studying the acute toxicity, which estimates the lethal dose (LD50) (Ahmed and Azmat 2014; Bertella et al. 2018). In the present study, the toxicity investigation of FSEO was performed in mice and revealed a value of LD50 of 4.9085 ± 0.1213 g/kg body weight. According to the Hodge and Sterner scale (Svarc-Gajiae et al. 2009; Ahmed and Azmat 2014), FSEO can be classified as mildly toxic since LD50 is between 500 and 5000 mg/kg. Although Mills et al. (2006) reported that LD50 of fennel EO varies from 1.3 to 4.5 g/kg, our results were better than those (1038 ml/kg, 3.12 g/kg, 3.8–1326 mg/kg rat body weight) reported in the literature (Albert-Puleo 1980; Ostad et al. 2001; Ozbek et al. 2005; European Medicines Agency 2008). Garnero (1996) also cited a LD50 of 4.52 mL/kg (4.06–5.05 mL/kg) in the acute oral toxicity of fennel EO in rats and greater than 5 g/kg in the acute dermal toxicity in rabbits. Several research works were devoted to studying the acute toxicity of EO of plant origin. The present study has shown that Algerian FSEO is safer than most of those reported for Lebanese *Prangos Asperula Boiss* (LD50 of 1.05 ml/kg) (Hilan et al. 2009), Brazilian EO (LD50 of 0.5 g/kg) of *Mesosphaerum sidifolium* (L'Hérit) (Rolim et al. 2017), Algerian *Thymus Fontanesii Boiss* (LD50 of 0.885 ± 0.08 g/kg) (Mouhi et al, 2017), and Algerian *Artemisia herba-alba* (LD50 of 0.615 g/kg) (Bertella et al. 2018).

Antioxidant activity of essential oils of Algerian wild fennel seeds

While DPPH free radical scavenging method could assess the in-vitro antioxidant activity of FSEO, BHT was used as a positive control. IC50 values were evaluated graphically (Fig. 3) and reordered in Table 6. BHT positive control showed better antioxidant capacity with IC50 value of

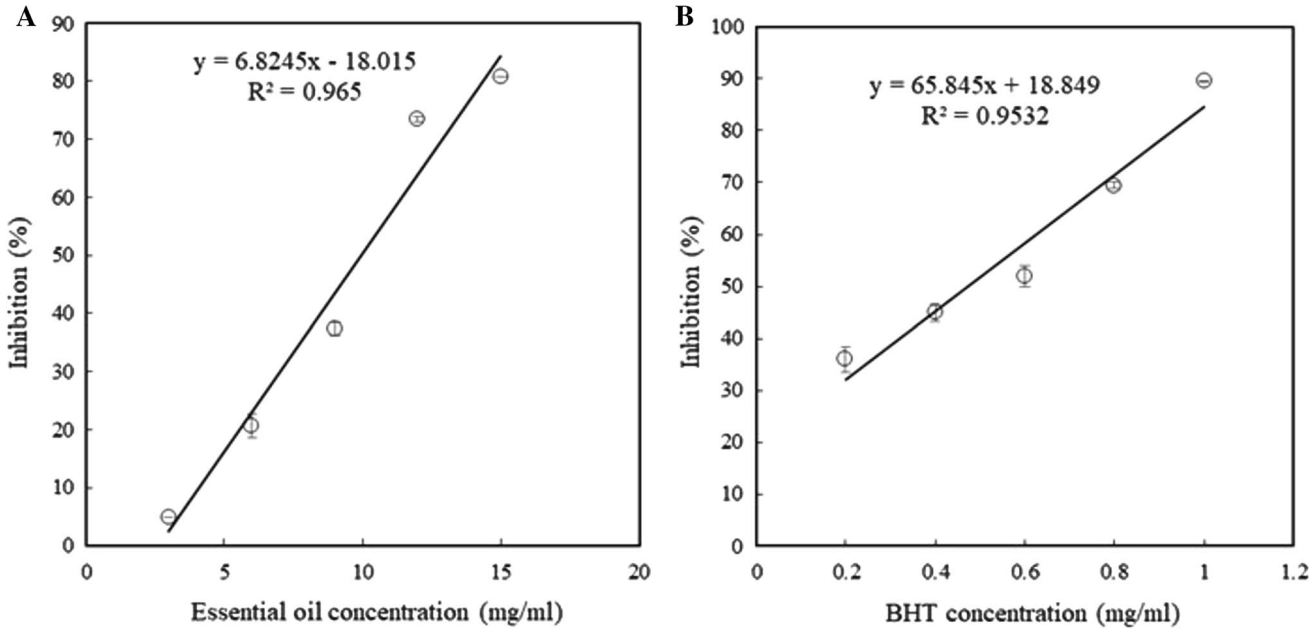


Fig. 3 Antioxidant capacity of Algerian wild fennel essential oil and BHT versus the concentration

Table 6 IC50 values of Algerian wild fennel essential oil and standard antioxidant

Sample	IC50 (mg/mL)
Essential oil	9.9658 ± 0.057
BHT	0.4570 ± 0.0456

0.4570 ± 0.0456 mg/mL compared to that of FSEO with IC50 value of 9.9658 ± 0.057 mg/mL. This finding concord with previous studies which reported that synthetic antioxidants have a better antioxidant capacity (De Marino et al. 2007; Anwar et al. 2009; Abdellaoui et al. 2017). Our result was comparable to those reported by Abdellaoui et al. (2017) (10.62 ± 0.33 mg/mL) for Moroccan wild FSEO, but better than that obtained (13.08 ± 0.34 mg/mL) for the same cultivated species, and those cited for Egyptian FSEO (15.33 mg/mL) by Shahat et al. (2011). As well as for the aerial part (15.6 mg/mL) of Tajikistan fennel Khammassi et al. (2018) have reported an IC50 value ranging from 12 to 38.13 mg/mL for Tunisian wild SEO. Ahmed et al. (2019) noted IC50 of 15.66 mg/g and 141.82 mg/g, for the EO of Chinese and Egyptian sweet fennel seeds, respectively. However, Anwar et al. (2009) reported a strong antioxidant capacity (10.62 ± 0.33 µg/mL) from FSEO from Pakistan.

Many ongoing works on the antioxidant capacity of EO from fennel show a considerable variation that the authors justify by several factors such as the origin, the climatic conditions, the method, the duration of extraction, and the chemical composition of EO (Abdellaoui et al. 2017; Ahmed et al. 2019; Yaldiz et al. 2019). The study of the antioxidant activity of EO has taken a great interest of searchers to replace synthetic antioxidants used in the food and pharmaceutical industries. The present study encourages the interest in valuing the Algerian wild FSEO as a natural antioxidant that can replace synthetic antioxidants. It is worth highlighting that these herbal-based substances are safer, more accepted by consumers, and sometimes more practical.

Antimicrobial activity of essential oils of Algerian wild fennel seeds: Qualitative study of antimicrobial activity

The disk diffusion method is a method to highlight the antimicrobial capacity against the tested microbial strains. Figure 4 illustrates the results, and Table 7 highlights the sensitivity degrees of the tested strains against FSEO. We found that yeasts were more sensitive to FSEO than bacteria. Likewise, the dose used had a more significant impact on the diameter of the inhibition zone for yeasts than for bacteria. Elsewhere, some authors reported that the diameter of the inhibition zone depended on the EO dose. Both fungal strains were susceptible for 15 µl and 20 µl of FSEO. They registered inhibition zones of 20.1 ± 0.45 mm and 26 ± 0.375 mm with *Candida albicans,* 15.9 ± 1.2 mm and 27.083 ± 1.292 mm with *Saccharomyces cerevisiaes*, respectively. However, the gram-positive *Bacillus subtilis* strain was more sensitive than the moderately sensitive, gram-negative *Escherichia coli* strain, while *Pseudomonas aeruginosa* was resistant to the tested doses. In general, gram-negative strains are more resistant than gram-positive

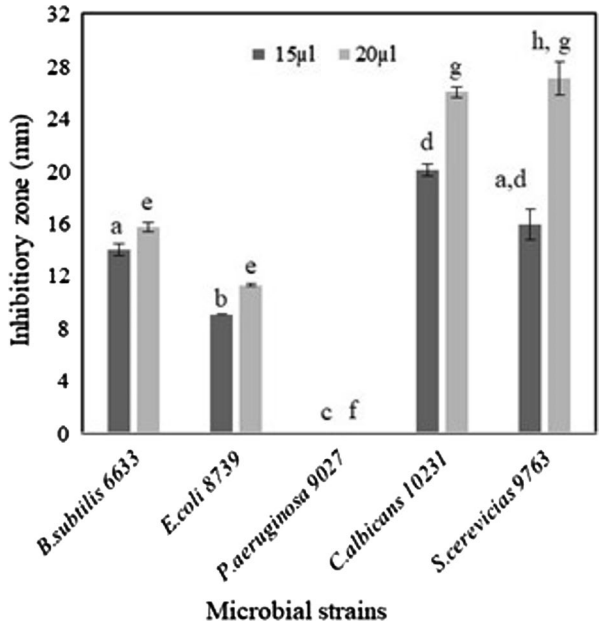


Fig. 4 Inhibition zone of fennel seeds essential oil on microbial strains (Means within the same dose noted by the same letter are not significantly different ($p > 0.05$) according to Tukey Post hoc multi comparison)

Table 7 Sensibility of microbial strains against fennel seeds essential oil

EO dose	*B. subtillis*	*E. Coli*	*P. aerugi-nosa*	*C. albicans*	*S. cerevi-ciaes*
HE (15 µL)	+ +	+	0	+ + +	+ +
HE (20 µL)	+ +	+	0	+ + +	+ + +

0: not sensitive; +: moderately sensitive; + +: sensitive; + + +: very sensitive

bacteria (Saviuc et al. 2012). Mashareq et al. (2016) reported the resistance of *Pseudomonas aeruginosa* to the EO (91.8% anethole) from fennel. This bacterium is known for its resistance because of its membrane's hydrophilic nature, which prevents the penetration of EO (Ilić et al. 2019). Our results agree with those found by A. Shahat et al. (2011) and Ilić et al. (2019), who noted significant antifungal activity of fennel EO against *Candida Albicans*. Ozken et al. (2006) showed variable fungistatic activity depending on the EO dose of bitter Turkish fennel. Boumahdi et al. (2020) also noted this finding by studying the same microbial strains with Algerian *Pimpinella anisum* essential oil.

Likewise, FSEO shows significant antifungal capacity against three fungal strains (*Alternaria alter, Fusarium oxysporum, and Aspergillus flavus*) (Javed et al. 2012). On the other hand, our results were lower than those found by Diao et al. (2014) for the antibacterial activity of fennel EO.

Quantitative study of antimicrobial activity

The disk diffusion method results showed that only fungal strains were sensitive to FSEO. This observation is crucial for determining the MIC and MFC for these strains. Table 8 presents the results with similar values of MIC (0.25%) and MFC (1%) for both tested fungal strains. The CMF/MIC ratio values, which do not exceed 4, can organize the fungicidal effects. Our results were higher than that found for *Candida albicans* (MIC of 0.5 and 0.4%) by Hammer et al. (1999) and by Gulfraz et al. (2008) for SEO, respectively. Moreover, Roby et al. (2012) and Ilić et al. (2019) noted for this strain a MIC of 10 µg/mL and 25 µg/mL with FSEO. The study of the antifungal activity of fennel EO against ten clinical strains of *Candida albicans* type showed a significant sensitivity of the strains which were resistant to fluconazole or sensitive to dependent doses (Bassyouni et al. 2018). Likewise, Kammasi et al. (2018) have reported a fungicidal activity of Tunisian wild fennel. The antimicrobial activity of EO depends on many factors, such as the method used, microbial strains, and chemical composition (structure and functional groups). Some authors (Javed et al. 2012, Bassyouni et al. 2018, and Ilić et al. 2019) reported that the antimicrobial activity of fennel EO was linked to anethol and/or its isomer and other compounds as fenchone, estragole, and limonene. Moreover, Senatore et al. (2013), Bertellla et al. (2018), and Ilić et al. (2019) also cited the presence of mono and sesqui-terpene hydrocarbons without neglecting synergistic interactions effects of minor compounds. Although the mechanism of their actions was not well studied, it may be due to the lipophilic character of EOs, which make them able to penetrate the cell membrane and mitochondria, causing structural damage enhancing permeability. That results in stopping the cell's biological metabolism and death (Khammassi et al. 2018; Ilić et al. 2019).

Conclusion

EO extraction from Algerian wild fennel seeds and umbels by hydrodistillation has shown that the seeds were richer in EO, which agrees with that reported by previous studies. Mathematical modeling of FSEO kinetic extraction

Table 8 MIC and MFC of Algerian fennel seeds essential oil on fungal strains

Fungal strains	MIC (%)	MFC (%)	MFC/MIC
Candida albicans ATCC 10231	0.25	1	4
Saccaromyces cereviciaes ATCC 9763	0.25	1	4

shows good results explaining the extraction phenomena. GC–MS analysis revealed new chromatographic profiles for the two studied organs. Fenchone (83.63%) and limonene (8.695%) are the major compounds of the FSEO, while α-pinene (23.513%), β-pinene (1.386%), Myrcene (1.994%), α-phellandrene (3.684%), o-cymene (18.309%), sylvestrene (8.869%), fenchone (13.637%), carvacrol (6.599%) and Endo-fenchyl acetate (13.165%) are the major compounds of FUEO. The FSEO was non-toxic with LD50 of 4.9085 ± 0.1213 g/kg and showed a good antioxidant capacity (IC50 of 9.9658 ± 0.057 mg/mL), matching previous studies. Likewise, a good antimicrobial activity has been noted, especially against fungal species. Finally, the present study results highlight the interest in valuing the Algerian wild fennel to replace the synthetic antioxidants used in industries (pharmaceuticals, cosmetics, and food) and other antimicrobial agents that present resistance problems developed by pathogenic microorganisms in hospitals and other environments. As a perspective of the whole study, which gave a new chromatographic profile of fennel EO, we plan to extend the future investigations and add to the present work other Algerian varieties from other regions, including very severe desert areas. We plan to perform a genetic identification of the fennel samples and a biological valorization of fennel EOs rich in interesting chemical compounds.

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Declarations

Conflict of interest We do not have any conflict of interest.

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