



(RESEARCH ARTICLE)



Phytochemical and microbiological study of the essential oil of *Deverra scoparia* from the Algerian Sahara

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Abstract

The work focuses on the phytochemical study of a species from the *Apiaceae* family: *Deverra scoparia* called wild fennel; collected at Ahaggar National Park in southern Algeria as well as a review of its biological activity: antimicrobial.

After extracting the essential oil from its ripe fruits, the chromatographic analysis is carried out:

Deverra scoparia displayed an intermediate value of 2.7%.

The antimicrobial activity of the essential oil is sought: It reports a moderate antibacterial and antifungal power.

This bioactivity is mainly due to α -pinene, which is partly responsible for the antimicrobial activity.

Keywords: *Deverra scoparia*; Essential oil; Antimicrobial activity; α -pinene; Ahaggar

1. Introduction

Deverra scoparia, also known as Wild Fennel of Ahaggar, is a perennial plant that forms large tufts and reaches a height of 40 to 80 cm. The leaves are reduced to scales along the branches and emit a pleasant odor. It is an endemic species of North Africa, common in the northern part of the Sahara and rare further south. In Ahaggar, it is found at altitudes ranging from 1000 to 2600 meters, in rocky riverbeds and on rocky slopes and ravines of the mountains. [1,3].

Deverra scoparia It is used by the population of Ahaggar to treat renal colic (kidney stones), and it is effective against back pain. The dried stems are used in the preparation of powders against snake bites, and when infused, it aids digestion. [2,3].

Thanks to its richness in flavonoids, in combination with Cymbopogon, it acts as a diuretic due to its flavonoids. [4]. The flowers, when infused, are used as a febrifuge and in the treatment of colds. It is good for grazing by goats and camels. [2,5]. The toxicity of *Deverra scoparia* increases during flowering, which makes it avoided by herbivores, and this toxicity seems to be related to the presence of alkaloids. [6].

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Figure 1 *Deverra scoparia* (Coss. et Dur.), general appearance (D. Boukhalfa)

2. Material and methods

2.1. Plant material

For this study, several samples of mature fruit of the plant were collected in Oued Tamanrasset over several years, from September to November, in the Ahaggar region at an altitude of 1385 meters.

The identification of the plant was carried out according to the new flora of Algeria and southern desert regions (Quezel P. and Santa S., 1977), the flora of Sahara (Ozenda P., 1983), by Professor D. Boukhalfa, Mr M. Belghoul (biologist, ONPCA), and confirmed by Mr. H. Abdelkrim, professor of botany (ENSA d'El Harrach).

2.2. Microbial material

The strains used are reference strains (American Type Culture Collection : ATCC), sensitive. All strains are pure and sourced from the collection of the microbiology laboratory at the Center for Research and Development (CRD), SAIDAL.

Bacterial strains were maintained by streaking on nutrient agar favorable for their growth for 24 hours, in the dark and at 37 °C. The only fungal strain tested, *Candida albicans*, was cultured on Sabouraud nutrient medium for 24 hours at 37 °C.

Table 1 Bacterial and fungal strains tested

Names of strains	N° ATCC	Forms	Taxon
<i>Staphylococcus aureus</i>	6538	CG+ Aerobic / Facultative anaerobic	<i>Micrococcaceae</i>
<i>Bacillus subtilis</i>	9372	BG+ Strict aerobe	<i>Bacillaceae</i>
<i>Pseudomonas aeruginosa</i>	9027	BG- Strict aerobe	<i>Pseudomonadaceae</i>
<i>Escherichia coli</i>	4157	BG- Aerobic / Facultative anaerobic	<i>Enterobacteriaceae</i>
<i>Candida albicans</i>	24433	yeast	<i>Cryptococcaceae</i>

2.3. Physicochemical equipment:

2.3.1. Refractometer

The refractometer used for determining the refractive index of all the essential oils of the studied drugs is a CARL ZEISS refractometer with reference number 89717.

2.3.2. Hydrodistillation apparatus:

Standardized apparatus for essential oil extraction in accordance with the European Pharmacopoeia 8th edition.

To prevent any contamination, the hydrodistillator was first washed with hot water, followed by absolute alcohol, toluene, and finally acetone (European Pharmacopoeia).

2.3.3. Equipment for physicochemical analysis :

The gas chromatograph used in this study is a Hewlett-Packard (HP) Palo-Alto CA, USA (Agilent technologies) 6800 plus.

- **Injector:** Split-splitless set at 250 °C, injection mode: Split 50:1. The sample is introduced with a microsyringe.
- **Column:**
 - Type : Hewlett Packard-5MS, non polar.
 - Dimensions : length 30 m, internal diameter: 0.25 mm, film thickness 0.25 µm.
 - Stationary phase : 5 % phenyl, 95 % dimethylpolysiloxane.
- **Detector :** The detector used is a mass spectrometer or Mass Spectrometer Detector (MSD): HP (Agilent technologies) MSD 5973, triple quadrupole mass filter (QQQ).

2.4. Extraction and quantification of essential oils :

After extraction of the essential oil, quantification is directly performed on the apparatus, and the correlation for 100 g of dried drug gives: X ml / 100 g of dried plant.

The essential oil is stored in tinted glass bottles, protected from heat and light.

Table 2 Operating conditions

Dry Wight	Solvent (ml)	Extraction duration
20	Water+ glycerin (200+100)	04 hours

2.5. Physical clues

2.5.1. Refractive index

The refractive index at 20 °C and density are determined by methods conforming to the AFNOR standards, 2011 [7]. The sampling is represented by pure essential oils, not damaged or modified during transportation or storage. The sample for testing is maintained at a temperature of 20°C until the end of the operation [8].

2.5.2. Relative density

This is the ratio of the mass of a certain volume of an essential oil at 20 °C to the mass of an equal volume of distilled water at 20 °C [7].

2.6. Chemical analysis of the essential oil

This is a separation by gas chromatography coupled with mass spectrometry (GC/MS):

Operating conditions of the GC/MS analysis :

- Injector: Set at 250 °C, injection mode: Split 50:1. Injected volume: 0.2 µl.
- Analysis mode : Scan (from 34 to 450)
- Solvent used : Hexane, solvent delay: 4 min
- Interface temperature: 280 °C
- Ionization type : Electron impact
- Filament intensity: 70 eV
- Mass analyzer type : Quadrupole

- Quadrupole temperature: 150 °C, Source temperature: 230 °C
- Oven temperature: Programmed at 60 °C (8 min), at a rate of 2°C/min up to 250 °C, for 10 minutes.
- Carrier gas: Helium purity: N 6, Carrier gas flow rate: 0.5 ml/min.

Each sample of essential oil is injected three times, as well as the internal calibration solution containing a mixture of hexanes.

2.7. Methods for Determining Antimicrobial Activity

The microbiological assays used in our study aimed to determine the inhibitory effect of essential oils against sensitive reference microbial strains.

The agar diffusion method using sterile cellulose discs impregnated with essential oils, known as aromagrams, [9] is the most commonly used method. It is a qualitative technique used to evaluate the antimicrobial activity by determining the inhibitory effect of essential oils against sensitive reference microbial strains. The agar diffusion method has been previously used by several researchers [10].

The strains were classified as resistant, moderately sensitive, sensitive, highly sensitive, and extremely sensitive based on the diameter of the inhibition zone, as follows:

- Resistant strain (-: or $D \leq 6\text{mm}$)
- Sensitive strain (+: or $9\text{mm} \leq D \leq 14\text{mm}$)
- Highly sensitive strain (+: or $15\text{mm} \leq D \leq 19\text{mm}$)
- Extremely sensitive strain (+++: or $D \geq 20\text{mm}$), according to criteria defined in the literature [11].

It is worth noting that all tests, both fungal and bacterial, were repeated three times. Positive controls (Gentamycin 20 mg/ml) and negative controls (Physiological saline) were also prepared.

3. Results and discussion

3.1. Physical Clues

Table 3 Yield, refractive index, and relative density

Plant	Average yield (ml/100gps)	IRn ^{x20}	Density g/ml
<i>Deverra scoparia</i>	2.8 ± 0.2	1.4935 ± 0.0005	0.9598

3.2. Results of gas chromatography-mass spectrometry (GC-MS)

This analysis gave the following chromatographic profile

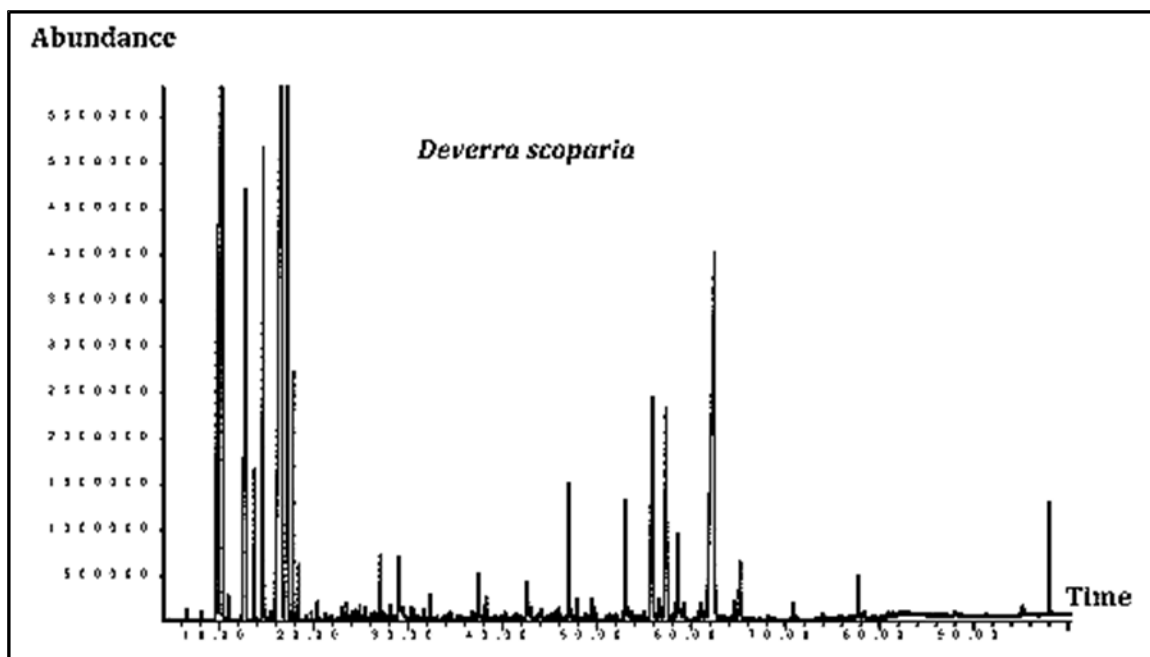


Figure 2 Chromatographic profile of the extract of the essential oil of *Deverra scoparia*

Table 4 Chemical composition of the essential oil of *Deverra scoparia*

N°	Identified compounds	TR	IR	%
01	α - thuyen	9.78	928	5.04
02	α -pinen	10.30	937	17.37
03	camphen	10.94	947	0.19
04	sabinen	12.57	974	1.91
05	β -pinen	12.77	977	3.82
06	β -myrcen	13.74	994	1.30
07	l-phellandren	14.69	1008	5.27
08	O-cymen	14.89	1011	0.11
09	α -terpinen	15.39	1018	0.11
10	dl-limonen	16.59	1034	20.35
11	β -ocimen	17.24	1043	6.75
12	α -ocimen	17.84	1052	2.19
13	γ -terpinen	18.41	1060	0.42
14	α -terpinolen	20.42	1088	0.15
15	neo-allo-ocimene	23.53	1131	0.16
16	cyclopentane, 1-ethenyl-3-methylene	25.41	1154	0.10
17	1-terpinen-4- ol	27.10	1181	0.64
18	α -terpineol	28.20	1196	0.14
19	trans-(+)-carveol	30.31	1226	0.12
20	p-cymol	30.51	1229	0.10

21	carvacrol	36.90	1322	0.12
22	Methyl eugénol	42.65	1409	0.50
23	trans-caryophyllen	43.05	1416	0.14
24	germacrenD	47.04	1480	1.37
25	germacrenB	47.89	1494	0.24
26	α -Farnesen	48.74	1508	0.05
27	δ -Cadinene	49.57	1523	0.18
28	(+) spathulenol	53.06	1582	1.30
29	(-)-Caryophyllene oxide	53.32	1586	0.14
30	Dillapiole	55.91	1632	3.35
31	Spathulenol	56.55	1643	0.22
32	τ -Muurolol	56.78	1648	0.12
33	β -Eudesmol	57.37	1658	3.47
34	N. Butylidènephtalide	58.61	1680	12.95
Total (%)				90.39

3.3. Assay of antimicrobial activity

Deverra scoparia essential oil has antimicrobial activity on the following bacterial species : *S. aureus* ATCC 6538 and *Bacillus subtilis* ATCC 9372, this activity is weak on *E. coli* ATCC 4157 and null on *P. aeruginosa* ATCC 9027, the antifungal activity of this essential oil is moderate for *Candida albicans* ATCC.

Table 4 Antimicrobial activity of the EO of *Deverra scoparia*

Inhibition diameter / Microorganisms	Essential oils of <i>Deverra scoparia</i> (diameter in mm)	Control (-) (distilled water)	Gentamycin (20 mg/ml)
<i>P. aeruginosa</i> ATCC 9027	< 9	-	37
<i>E. coli</i> ATCC 4157	11	-	25
<i>B. subtilis</i> ATCC 9372	17	-	34
<i>S. aureus</i> ATCC 6538	20	-	36
<i>C. albicans</i> ATCC 24433	16	-	25

4. Discussion

4.1. Yield and Physical Indices

4.1.1. Yield

The essential oil of this species is light yellow in color, with a characteristic and pleasant odor. It yields a highly interesting amount (2.8 ± 0.2 ml/gram of dry matter) after hydrodistillation, compared to that of *Deverra scoparia* from Biskra (0.25 %).

4.1.2. Physical Clues

Relative density at 20 °C : 0.9598, indicating that the essential oil is very light compared to water. Refractive index at 20 °C : 1.4935 ± 0.0005 , which is higher than that of water at 20 °C and olive oil at 20 °C.

4.2. Gas Chromatography-Mass Spectrometry:

Thirty-four compounds were identified in the essential oil of *Deverra scoparia* from Ahaggar, accounting for a total of 90.39 %. The essential oil of *Deverra scoparia* is rich in monoterpenes (79.81%), with 65.24 % being hydrogenated monoterpenes (MH). The dominant compound is dl-limonene (20.35 %), followed closely by α -pinene (17.37 %), β -ocimene (6.75 %), β -pinene (3.82 %), l-phellandrene (5.27 %), α -thujene (5.04 %), α -ocimene (2.19 %), sabinene (1.91 %), and β -myrcene (1.30 %).

Oxygenated monoterpenes (MO) make up 14.57 %, with N-butylidene phthalide (12.95 %) being the major compound, followed by α -terpineol (1.11%). Sesquiterpenes account for only 10.58% of the total, with 8.60 % being oxygenated sesquiterpenes (SQO)(dillapiole (3.35 %), β -eudesmol (3.47 %), and spathulenol (1.30 %)), and 1.98 % being hydrogenated sesquiterpenes(SQH) (germacrene B and D (1.61%)).

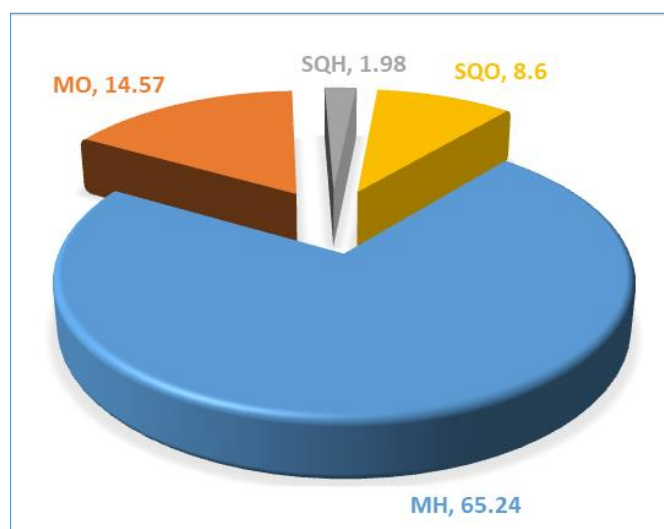


Figure 3 Chemical classes coposant essential oil of *Deverra scoparia*

The gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil of *Deverra scoparia* confirmed its richness in hydrogenated monoterpenes (MH), with dl-limonene being the major compound that contributes significantly to the plant's aroma. It is followed by α -pinene, which is partly responsible for the antimicrobial activity of the essential oil, cis-ocimene (6.75 %), l-phellandrene (5.27%), α -thujene (5.04%), and β -pinene (3.82 %) are also important constituted.

This oil also contains N-butylidene phthalide (12.95 %), and 10.58% of sesquiterpenes, with 8.60% being oxygenated sesquiterpenes ((SQO) such as dillapiole (3.35 %) and β -eudesmol (3.47 %)). Sesquiterpenes, on the other hand, represent only 10.58 % of the oil, with dillapiole being considered a typical compound of the Apiaceae family.

N-butylidene phthalide, which represents a significant content, is an aromatic bicyclic lactone known for its ability to drain the organs such as the liver, kidneys, and intestines, contributing to detoxification of the body.

4.3. Microbiological activities:

The essential oil of *Deverra scoparia* from Hoggar shows variable antibacterial and antifungal activities against certain reference strains. *Staphylococcus aureus* (20mm), *Bacillus subtilis* (17mm), and *Candida albicans* (16mm) demonstrate varying degrees of sensitivity. The activity is moderate against *Escherichia coli* and absent against *Pseudomonas aeruginosa*.

These results are consistent with those obtained from the same species in other regions of Algeria. For example, the essential oil from populations in Biskra has shown significant antimicrobial activities against both Gram-positive and Gram-negative bacteria (*Shigella sp. and Staphylococcus aureus*), as well as against the yeast *Candida albicans*.

5. Conclusion

From a biogeographical perspective, this study primarily focused on a species of Mediterranean origin, with some tropical origins. The unique environment of the studied plant has significantly influenced its chemical composition. The chemical analysis has confirmed the expected chemotype.

The study of antimicrobial activity has revealed that the extracted essential oil has variable antibacterial activity, which may potentially vary depending on the plant's vegetative stage.

Based on our study, we can conclude that the traditional uses of wild fennel from Ahaggar by the local population are justified.

Compliance with ethical standards

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Disclosure of conflict of interest

The author and all co-authors declare that they have no conflicts of interest in relation to this document, and the material described is not in publication or intended for publication elsewhere.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

Statement of informed consent

The studies presented in this manuscript do not involve any information on individuals.

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