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Chemical composition, antibacterial and antioxidant activities of essential oils from flowers, leaves and aerial parts of Tunisian *Dittrichia Viscosa*

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ABSTRACT

The objective of this work was to determine the chemical composition, the antioxidant and antibacterial activities of the essential oils (EOs) extracted by hydrodistillation from different organs of the *D. viscosa*: flowers, leaves and aerial parts. The main compounds identified by GC/MS are oxygenated sesquiterpenes. Among these compounds, (*E*)-nerolidol (40.7%) is the most abundant constituent of flowers' essential oil while caryophyllene oxide (9.9%), isolongifolan-7-α-ol (10.3%) and α -eudesmol (9.1%) are the major constituents of the leaves' essential oil. The presence of these compounds in the aerial parts' essential oil is solely due to those of the flowers and leaves that constitute these aerial parts. The volatile extracts showed antioxidant effects with IC_{50} values ranging between 9.25 and 9.75 $mg.mL^{-1}$. On the other hand, EOs showed antibacterial effects on both Gram-positive and Gram-negative bacteria. The highest activity was obtained with flowers' essential oil against *Enterococcus faecalis* and *Escherichia coli*.

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KEYWORDS
Dittrichia viscosa; essential oils; hydrodistillation; GC-MS; antioxidant activity; antibacterial activity

1. Introduction

For a long time, natural remedies and especially those based on medicinal plants have been the main or even the only medicine used in Tunisia. The Mediterranean and semi-arid climate of this country favors the growth of various types of wild plants whose valorization is under-exploited. Some of them, such as *Artemisia campestris L., Lavandula stoechas L., Origanum glandulosum, Pelargonium graveolens* ., are known for their anti-diabetic effects in traditional medicine (1) while others have been recognized as having anti-bacterial effects (*Santolina chamaecyparissus L.*) (2) or herbicide effects (*Pinus nigra*) (3) . The valorization of all these natural resources is mainly based on the extraction of volatile molecules, called "essential oils" whose composition determines their usefulness for medicinal, cosmetic or food applications.

One of the most common wild plants in the Mediterranean basin is the genus *Dittrichia* syn. *Inula* that belongs to the *Asteraceae* family. Four species of this genus are present in Tunisia: *Dittrichia viscosa (L.), Dittrichia graveolens (L.), Dittrichia crithmoides (L.)* and *Dittrichia montana (L.)* (4). The most abundant species, *Dittrichia viscosa (D. viscosa)*, is a perennial plant with a strong smell of camphor. It flowers in late summer and early autumn (October) and presents at the top of the stem numerous yellow flowered heads. It is found in rocky soils and on the roadsides where it can reach 50 cm to 1 m in height. The presence of *D. viscosa* in the olive groves is appreciated because it is known to house a useful and parasitic species of the olive fly (*Eupelmus urozonus*) (5) . On the other hand, *D. viscosa* has always been used in traditional medicine for its antidiabetic (6), antibacterial (7), antioxidant (8), cytotoxic (8) and anti-inflammatory (9) properties.

Some studies have been conducted to identify the chemical composition of volatile extracts from *D. viscosa* collected from Turkey (10), Italy (11), Algeria (12) and France (13) but none in Tunisia. On the other hand, all the studies carried out on *D. viscosa* showed great differences in chemical composition according to the area where the species was collected. Thus, the main objective of this work is to present for the first time the chemical composition of essential oils extracted from flowers, leaves and all the aerial parts of the species *D. viscosa*. A comparative study will be made with the literature results obtained for the same species but coming from different geographical areas.

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In the framework of the valorization of the Tunisian flora, the biological activities of these essential oils extracted from the different organs of *D. viscosa*, will also be studied.

2. Experimental

2.1. Plant material

Dittrichia viscosa (AST/Ditt.vis, 01012/2018) has been selected and identified by Dr. Ridha El Mokni, a researcher in botany at the faculty of pharmacy of Monastir, Tunisia. This plant was harvested in November 2015 in an olive grove in Monastir, a central-eastern Tunisian city. The leaves, flowers and all the aerial part were dried separately in the dark and at room temperature. The dry materials were then crushed and weighed before the essential oil extraction.

2.2. Extraction of essential oils

The dried leaves, flowers and all the aerial parts were hydrodistillated in a Clevenger-type apparatus for 3 h. The volatile extracts were collected by decantation, dried over anhydrous sodium sulfate, filtered and stored at −4°C until analysis.

2.3. GC-FID and GC-MS analysis

GC-FID analyses of the extracts were performed using a gas chromatograph (Agilent 7890A, Palo Alto, CA, USA), equipped with a 30 m × 0.25 mm i.d. with 0.25 µm stationary film thickness DB-5 capillary column (Agilent J&W) and a flame ionization detector (FID). The following temperature program was used: from 60°C to 246°C at rate of 3°C min^{-1} and then held at 246°C for 20 min (total analysis time, 82 min). Other operating conditions are the following: carrier gas helium (purity ≥ 99.9999% – Air Liquide Italy); flow rate, 1.0 $mL.min^{-1}$; injector temperature, 250°C; detector temperature, 300°C. Injection of 1 µL of diluted sample (1:100 in hexane, w/w) was performed with 1:10 split ratio, using an autosampler (Agilent, Model 7683B). GC-MS analyses were carried out using a gas chromatograph (Agilent 6890N) equipped with a 30 m × 0.25 mm i.d. with 0.25 µm stationary film thickness HP-5ms capillary column (Agilent J&W) coupled with a mass selective detector having an electron ionization device, EI, and a quadrupole analyzer (Agilent 5973). The temperature program was the same used for GC-FID. Other chromatographic operating conditions are the following: carrier gas, helium (purity ≥ 99.9999%); flow rate 1.0 $mL.min^{-1}$; injector temperature, 250°C. Injection of 1 µL of diluted sample (1:100 in hexane, w/w) was performed with 1:20 split ratio, using an autosampler (Agilent, Model 7683B). The MS conditions were as follows: MS transfer line temperature, 240°C; EI ion source temperature, 200°C with ionization energy of 70 eV; quadrupole temperature, 150°C; scan rate, 3.2 $scan.s^{-1}$ at mass-to-charge, m/z, scan range from 30 to 480. To handle and process chromatograms and mass spectra was used the software MSD ChemStation (Agilent, rev. E.01.00.237).

Constituents of the samples were identified by comparing: mass spectra fragmentation patterns with those of a computer library (14) and linear retention indices (RI) based on a homologous series of C8-C26 n-alkanes with those reported in the literature (15).

2.4. Antioxidant activity

The antioxidant activity of *D. viscosa* essential oil was evaluated by 2,2- diphenylpicrylhydrazyl radical (DPPH°). The essential oils of *viscosa* flowers, leaves and aerial part were dissolved in absolute ethanol at concentration ranges of 1 to 10 $mg.mL^{-1}$. Then, 0.5 mL of each ethanolic solution was added to the same volume of DPPH ethanolic solution (0.1mM) (16). A negative control was prepared by mixing the ethanol with the DPPH ethanolic solution. Quercetin was used for comparison. Each mixture was homogenized and stored at room temperature in the dark for 30 min and then read at 517 nm using a spectrophotometer model T70 UV/VIS Spectrometer PG Instruments Ltd. The inhibition percentage (% PI) of DPPH radicals was calculated by the following formula (17):

$$\%PI = \frac{Abs_0 - Abs_i}{Abs_0} \times 100$$

where Abs_0 is the absorbance of the negative control (0.1 mM DPPH solution) and Abs_i is the absorbance of the samples.

The antioxidant activities of the essential oils were expressed by IC_{50} values which denote the concentration of samples required to scavenge 50% of the DPPH° radicals.

2.5. Antibacterial activity

2.5.1. Disc diffusion assay

Antibacterial activity, using the disk-diffusion assay, was evaluated against three Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27,853, *Escherichia coli* ATCC 35,218 and *Salmonella typhimurium* ATCC 1408) and one Gram-positive bacteria (*Enterococcus faecalis* ATCC 29,212). The essential

oils were diluted at a concentration of 5 mg.mL^{-1} in 5% DMSO solution. A few drops of Tween 80 were added to improve the solubility of the extracts.

Bacterial suspensions (10^6 CFU.mL^{-1}) were inoculated on the surface of Muller Hinton agar plates. Then, filter paper discs impregnated with 10 µl of the diluted extracts were placed on each Muller Hinton agar inoculated plate. The inhibition zone diameters around each disk were measured after 18 h of incubation at 37°C.

2.5.2. Minimal inhibitory concentration (MBC) assay

The minimal inhibitory concentration (MIC) of the tested samples was evaluated by the standardized micro-dilution method in Muller Hinton and adapted to multiwell microtiter plates (plate ELISA, 96 wells). Serial dilution of the essential oils was made in Muller Hinton broth (MH), ranging from 0.50 to 2.75 mg.mL^{-1}. Then, 10 µL of each microbial strain (10^6 CFU.mL^{-1}) were added to each well. The microtiter plates were incubated for 24 h at 37°C. The MIC value was defined as the lowest concentration of extract that had no macroscopically visible growth.

2.5.3. Minimal bactericidal concentration (MBC) assay

For the MBC determination, 10 µL of each well medium with no visible growth was poured on MH agar plates and incubated at 37°C for 24 h. MBC was defined as the lowest concentration for which 99% of the bacteria were killed.

3. Results and discussion

The essential oils obtained by hydrodistillation have a light yellow color with a very pleasant smell. The extraction yields were evaluated at 0.15%, 0.10% and 0.10% for flowers, leaves and the whole aerial part, respectively.

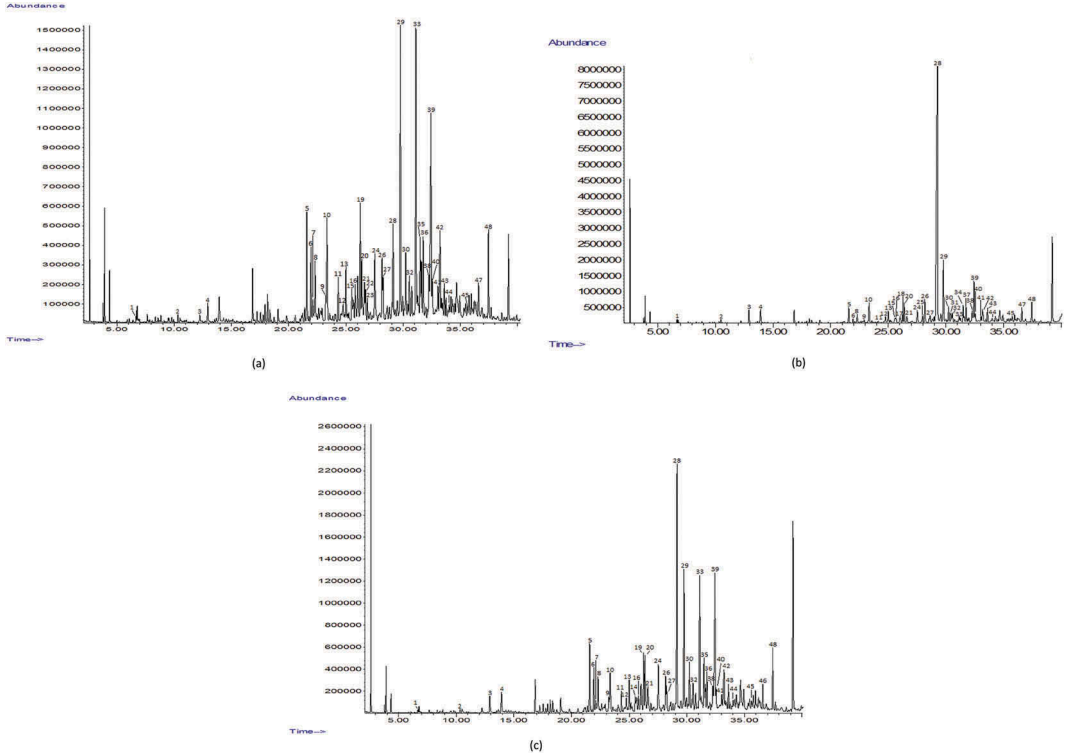

Figure 1. GC chromatograms of *D. viscosa* essential oil from leaves (a), flowers (b) and aerial part (c). Peak identification: **1** 1,8-dehydrocineole; **2** n-nonanal; **3** para-mentha-1,5-dien-8-ol; **4** α-terpineol; **5** α-copaene; **6** (*E*)-β-damascenone; **7** (*Z*)-β-damascenone; **8** 1-tetradecene; **9** α-cedrene; **10** (*E*)-caryophyllene; **11** aromadendrene; **12** geranyl acetone; **13** allo-aromadendrene; **14** cis-thujopsadiene; **15** β-chamigrene; **16** γ-muurolene; **17** β-selienene; **18** 10,11-epoxy-calamenene; **19** δ-selienene; **20** cis-β-guaiene; **21** α-muurolene; **22** epizonarene; **23** α-cuprenene; **24** δ-cadinene; **25** α-cadinene; **26** α-copaen-11-ol; **27** α-calacorene; **28** (*E*)-nerolidol; **29** caryophyllene oxide; **30** 1-hexadecene; **31** fokienol; **32** guaiol; **33** isolongifolan-7-α-ol; **34** 1-epi-cubenol; **35** muurola-4,10(14)-dien-1-β-; **36** gymnomitrone; **37** epi-α-cadinol; **38** cedr-8(15)-en-9-α-ol; **39** α-eudesmol; **40** α-cadinol; **41** 14-hydroxy-(*Z*)-; **42** 14-hydroxy-9-epi-(*E*)-; **43** ishwarone; **44** epi-nootkatol; **45** 8-α-11-elemodiol; **46** β-costol; **47** 13-hydroxy-valencene; **48** 1-octadecene.

3.1. Component identification

To our knowledge, the phytochemical study of *D. viscosa* essential oil growing in Tunisia is reported for the first time. Figure 1 (GC chromatograms of *D. viscosa* essential oil) and Table 1 gives the results of the GC/MS and CPG/FID analysis which identified forty-seven volatile compounds mostly classified as terpenoids. Thus, oxygenated sesquiterpenes account for more than 45.8%, 64.7% and 44.6% of volatile compounds from flowers, leaves and aerial parts, respectively.

Among these sesquiterpenes, (*E*) -nolidolidol was found mainly in the essential oils of the flowers at 40.7 against 2.8% in those of the leaves. In contrast, isolongifolan-7-α-ol and α-eudesmol, two other oxygenated sesquiterpenes, were mostly found in the essential oils of the leaves at 10.3% and 9.1%, respectively, whereas, in the flowers' essential oil, they were detected at only 0.6% and 3.7%, respectively. Caryophyllene oxide, also belonging to the family of oxygenated sesquiterpenes, has been found in similar amounts in both organs, in 6.0% and 9.9% of the essential oils of flowers and leaves, respectively. Figure 2 gives the chemical structures of the main essential oil compounds, i.e. (*E*)-nolidolidol, isolongifolan-7-α-ol, α-eudesmol and caryophyllene oxide. The presence of all these compounds in the essential oils of the aerial parts is solely due to the flowers and leaves that constitute it.

Table 2 presents, for comparison, the results of the literature dealing with the essential oil chemical composition of *D. viscosa* growing in different regions. This comparative study, which concerns only the composition of the aerial part essential oils, shows a slight similarity in the proportions of (*E*)-nerolidol in the Tunisian and French (13) *D. viscosa* essential oils estimated at 11.0 et 8.6%, respectively. On the other hand, none of the other major compounds found in the Tunisian plant, namely the caryophyllene oxide, isolongifolan-7-α-ol and α-eudesmol, is present in significant quantities in Turkish (10), Italian (11), Algerian (12) or French (13) plant of the same species.

Several factors are responsible for this variability in the chemical composition of essential oils of the same species. Among these factors: the environmental conditions (climate, nature of the soil, etc.), the conditions of harvest and conservation (harvest period, drying and grinding, etc.) and the extraction operating conditions (time, temperature, ratio liquid/solid, etc.). These factors influence the vegetative cycle of the plant and contribute to the chemical diversity of its volatile compounds.

Thus, the essential oil extracted from the Tunisian plant, and more precisely from its flowers, is very rich in (*E*)-nerolidol, since it is present at 40% of the total volatile constituents (Table 2). (*E*)-nerolidol is an odorant compound prized by the perfume industry (18) but some studies have shown that it may have anti-parasitic (19), anti-malarial (20), anti-mite (21) and cytotoxic (22) properties. A recent study has even highlighted the effect of

Table 1. Chemical composition of the essential oils from the leaves (LE), flowers (FL) and aerial parts (AE) of *D.viscosa*.

Peak number	Compound	RI (HP-5)	RI (Literature)	LE	FL	AE
1	1,8-dehydro-cineole	992	991	tr	0.2	tr
2	n-nonanal	1104	1100	tr	0.3	tr
3	para-mentha -1,5-dien-8-ol	1168	1170	tr	0.9	0.7
4	α-terpineol	1191	1188	1.1	1.4	1.2
5	α-copaene	1376	1376	3.2	1.2	2.8
6	(*E*)-β-damascenone	1384	1384	2.3	0.3	1.9
7	(*Z*)-β-damascenone	1388	1387	0.8	tr	tr
8	1-tetradecene	1392	1389	1.9	0.7	1.7
9	α-cedrene	1415	1411	0.7	tr	0.6
10	(*E*)-caryophyllene	1418	1419	4.0	1.9	2.4
11	aromadendrene	1443	1441	1.3	tr	0.8
12	geranyl acetone	1454	1455	tr	0.7	tr
13	allo-aromadendrene	1460	1460	1.5	1.0	1.3
14	cis-thujopsadiene	1472	1467	1.2	tr	0.9
15	β-chamigrene	1475	1477	tr	0.7	tr
16	γ-muurolene	1476	1479	1.5	0.5	1.0
17	β-selienene	1485	1490	tr	0.8	tr
18	10,11-epoxy-calamenene	1490	1491	-	0.6	-
19	δ-selienene	1490	1492	3.8	-	2.5
20	cis-β-guaiene	1493	1493	2.5	1.6	2.8
21	α-muurolene	1498	1500	1.6	0.8	1.8
22	epizonarene	1501	1501	1.0	tr	tr
23	α-cuprenene	1505	1505	0.6	tr	tr
24	δ-cadinene	1523	1523	2.8	1.2	3.0
25	α-cadinene	1535	1538	tr	0.8	tr
26	α-copaen-11-ol	1540	1540	1.9	2.2	1.7
27	α-calacorene	1542	1545	1.3	tr	1.2
28	**(*E*)-nerolidol**	1565	**1563**	**2.8**	**40.7**	**11.0**
29	**caryophyllene oxide**	1581	**1580**	**9.9**	**6.0**	**6.7**
30	1-hexadecene	1592	1589	2.2	1.5	2.2
31	fokienol	1597	1596	tr	1.1	tr
32	guaiol	1600	1600	1.5	1.1	1.5
33	**isolongifolan-7-α-ol**	1616	**1619**	**10.3**	**0.6**	**6.3**
34	1-epi-cubenol	1627	1628	tr	1.3	tr
35	muurola-4,10(14)-dien-1-β-	1627	1631	2.6	tr	2.3
36	gymnomitrone	1630	1632	1.9	tr	1.2
37	epi-α-cadinol	1641	1640	tr	0.4	tr
38	cedr-8(15)-en-9-α-ol	1648	1651	1.4	1.2	1.2
39	**α-eudesmol**	1653	**1653**	**9.1**	**3.7**	**7.9**
40	α-cadinol	1656	1654	1.3	0.7	1.1
41	14-hydroxy-(*Z*)-	1669	1667	tr	0.4	tr
42	14-hydroxy-9-epi-(*E*)-	1673	1669	0.9	1.4	0.6
43	ishwarone	1684	1681	1.0	1.1	1.1
44	epi-nootkatol	1695	1699	tr	0.4	tr
45	8-α-11-elemodiol	1745	1747	tr	0.6	0.8
46	β-costol	1769	1767	tr	Tr	1.2
47	13-hydroxy-valencene	1769	1768	1.2	1.2	tr
48	1-octadecene	1792	1790	2.7	1.6	2.8
	Sesquiterpenes hydrocarbons			27.0	10.5	21.1
	Oxygenated sesquiterpenes			45.8	64.7	44.6
	Oxygenated monoterpenes			4.2	3.5	3.8
	Others			6.8	4.1	6.7
	Total			**83.8**	**82.8**	**76.2**

RT*: Retention time, RI: Retention index determined on a HP5-MS fused silica column relative to a series of n-alkanes (C8-C26),
tr: trace, percentage lower than 0.1%

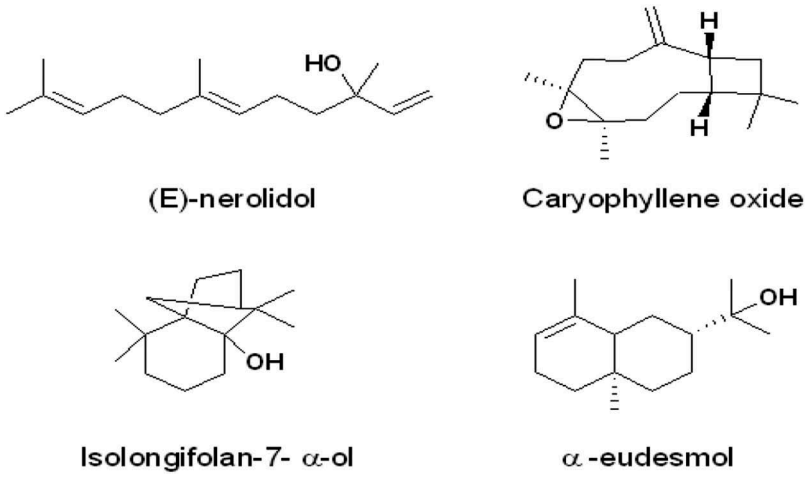

Figure 2. Chemical structure of the main essential oil constituents.

Table 2. Majority compounds in *D.viscosa* essential oil: a comparative study with literature.

	LE	FL	AE	AE	AE	AE	AE
Carboxyeudesma-diene	n.d	n.d	n.d	n.d	n.d	28.8%	62.4%
Linolenic acid	n.d	n.d	n.d	n.d	n.d	7.8%	n.d
Fokienol	n.d	n.d	n.d	21.1%	n.d	3.3%	n.d
(*E*)-nerolidol	2.8%	40.7%	11.0%	8.6%	1.5%	n.d	n.d
Caryophyllene oxide	9.9%	6.0%	6.7%	2.5%	1.5%	0.2%	n.d
Isolongifolan-7- α -ol	10.3%	0.6%	6.3%	n.d	n.d	n.d	n.d
α-eudesmol	9.1%	3.7%	7.9%	n.d	n.d	n.d	n.d
Eudesma-6-en-4- α-ol	n.d	n.d	n.d	6.2%	n.d	n.d	n.d
Borneol	n.d	n.d	n.d	n.d	25.2%	n.d	n.d
Bornyl acetate	n.d	n.d	n.d	n.d	19.5%	n.d	n.d
Isobrnyl acetate	n.d	n.d	n.d	n.d	22.5%	n.d	n.d
Monoterpene hydrocarbons				1.1%	4.8%		0.3%
Oxygenated monoterpenes	4.2%	3.5%	3.8%	6.5%	72.8%		
Sesquiterpenes hydrocarbons	27.0%	10.5%	21.1%	4.4%	2.2%	1.2%	3.1%
Oxygenated sesquiterpenes	45.8%	64.7%	44.6%	60.0%	10.2%	46.7%	72.2%
others	6.8%	4.1%	6.7%	2.7%			1.7%

n.d: not detectable, LE: Leaves, FL: Flowers, AE: Aerial parts

(*E*)-nerolidol and caryophyllene oxide (23) on some cancer cells. Caryophyllene oxide, which is present in all organs of the Tunisian plant at 6.0–9.9%, has been recognized by some researchers as having analgesic and anti-inflammatory activities (24).

3.2. Antioxidant and antibacterial activities

The antioxidant activity of volatile extracts from different organs of *D. viscosa* was evaluated in terms of inhibition concentration (IC_{50}). This concentration is deduced from the curve representing the inhibition percentage as a function of the essential oil concentration (Figure 3).

Table 3 gives the result of this evaluation, which shows that *D. viscosa* essential oils have low antioxidant capacities (9.3 to 9.8 $mg.mL^{-1}$) in comparison to that of the quercetin positive control (0.5 $mg.mL^{-1}$). In fact,

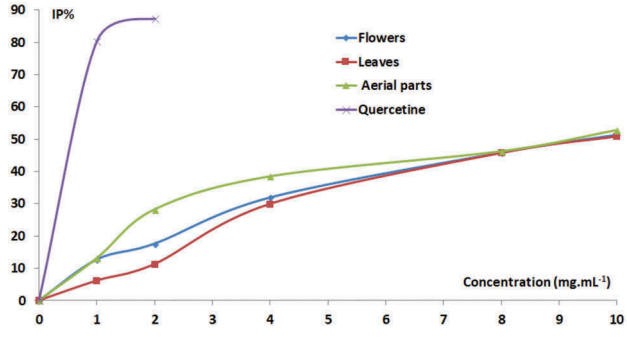

Figure 3. Antioxidant activity profile of *D. viscosa* essential oils.

Table 3. Antioxidant activity of *Dittrichia viscosa* essential oils.

sample	IC_{50} ($mg.mL^{-1}$)
Aerial parts	9.25
Flowers	9.50
Leaves	9.75
Quercetine	0.50

Table 4. Inhibition zone diameter for antibacterial activity of *Dittrichia viscosa* essential oils.

	Essential oil			Control
Microorganism	Leaves	Flowers	Aerial parts	Chloramphenicol
Gram-positive bacteria				
Enterococcus faecalis ATCC 29,212	12	17	-	11
Gram-negative bacteria				
Pseudomonas aeruginosa ATCC 27,853	-	-	10	13
Escherichia coli ATCC 35,218	8	12	12	11
Salmonella typhimurium ATCC 1408	-	-	-	12

Inhibition zone diameter (d) (mm): – inactive, moderate activity (d ≤ 10), interesting activity (10 < d ≤ 15), high activity (d > 15)

D. viscosa essential oils are rich in sesquiterpenes that reach 65–75% of the total volatile compounds (Table 1). Unfortunately, the sesquiterpenes are neither soluble in the reaction medium of the DPHH radical scavenging test nor capable of giving a hydrogen atom (25). On the other hand, the absence of phenolic derivatives, known for their primordial role as natural antioxidants (26,27), can also explain this low oxidizing activity.

The antibacterial activity of *D. viscosa* essential oils was evaluated against four bacterial strains: *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhimurium* and *Enterococcus faecalis*. The results presented in Table 4 show a significant anti-bacterial activity of the essential oil extracted from the flowers against *Enterococcus faecalis*, a gram-positive bacterium and against *Escherichia coli*, a gram-negative bacterium. On the other hand, Table 5 displays the minimum inhibition concentration (MIC) and the minimum bactericidal concentration (MBC) values for the flowers' essential oil against *Enterococcus faecalis* that were evaluated at 1 and 1.75 $mg.mL^{-1}$, respectively. Some literature studies have shown that (E)-nerolidol has an antibacterial activity against *Staphylococcus aureus* and *Echerichia coli* (28). As this sesquiterpenic compound is the main constituent of the flowers' essential oil (40%), it was expected to find an antibacterial effect of these oils against *Escherichia coli*.

Table 5. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *Dittrichia viscosa* essential oils.

	MIC ($mg.mL^{-1}$)			MBC ($mg.mL^{-1}$)		
Microorganism	Leaves	Flowers	Aerial parts	Leaves	Flowers	Aerial parts
Gram-positive bacteria						
Enterococcus faecalis ATCC 29,212	1.50	1.00	-	2.75	1.75	-
Gram-negative bacteria						
Pseudomonas aeruginosa ATCC 27,853	-	-	2.25	-	-	2.50
Escherichia coli ATCC 35,218	2.50	1.50	2.25	2.75	2.00	2.75
Salmonella typhimurium ATCC 1408	-	-	-	-	-	-

-: inactive

4. Conclusion

The chemical composition of *Dittrichia viscosa* essential oils extracted from different organs (flowers, leaves and the whole aerial parts) showed a high proportion of oxygenated sesquiterpenes and more particularly the (*E*)-nerolidol. More particularly, the flowers' essential oil were dominated by (*E*)-nerolidol (40%) which can explain their antioxidant and anti-bacterial potential. Thus, the antibacterial activities carried out on flowers' essential oil showed excellent results with respect to gram-positive and gram-negative bacteria. The minimum inhibition concentration and the minimum bactericidal concentration were evaluated at, respectively, 1.5 $mg.mL^{-1}$ and 2 $mg.mL^{-1}$ against *Escherchia coli*, and at, respectively, 1 mg/mL and 1.75 mg/mL against *Enterococcus faecalis*.

These results are promising but can be improved by the extraction of essential oil from fresh flowers that are probably richer in monoterpene compounds known for their high anti-oxidant and anti-bacterial potential.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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