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Antimicrobial Activities of Natural Volatiles Organic Compounds Extracted from *Dittrichia viscosa* (L.) by Hydrodistillation

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Abstract

The chemical characterization and antimicrobial activities of *Dittrichia viscosa* (L.) essential oils extracted by hydrodistillation technique from leaves and stems are reported in this work. *Dittrichia viscosa* (L.) samples were collected from the Bainem forest in the northwest part of Algiers (the capital). Gas chromatography-mass spectrometry (GC-MS) analytical method was employed to identify the oil's chemical composition. It was found that leaves are mainly composed of three major abundant composites, specifically, caryophyllene oxide (10.4%), fokienol (9.6%) and trans-nerolidol (7%). Moreover, the oil isolated from the stems was found to be chiefly composed of trans-totarol (18.1%), α-cedrol (16.7%), and ferruginol (16.6%). Additionally, antimicrobial activity tests were performed on the isolated essential oils using the zone of inhibition (agar disk-diffusion method) to determine the minimum inhibitory concentration (MIC) of four bacteria strains, mainly, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli*. The MIC values of leaves are found to range between 15 µg and 100 µg, whereas stems are found to exhibit MIC values ranging between 20 µg and 300 µg. Furthermore, antifungal susceptibility tests, which become important tools to dictate the treatment of fungal diseases, are conducted on two yeast strains: *Saccharomyces cerevisiae* and *Candida albicans*. The obtained antimicrobial results are correlated with the chemical composition findings of the essential oils from leaves and stem to determine the roles of the chemical composites on the antibacterial activity. Interestingly, the oil obtained from the leaves displayed a better inhibitory effect on (bacteria and yeast strains) in comparison with oil (stems). This difference in inhibitory effect can be attributed to the dominant existence of the oxygenated sesquiterpenes and trans-nerolidol compounds in leaves.

Keywords: *Dittrichia viscosa* (L.); Volatile Organic Compounds; Chemical Reactivity; Antibacterial Activity.

1. Introduction

Finding new antibiotics has gained extensive attention, taking into consideration that several existing drugs have become inefficient due to the timewise increase in antimicrobial resistance (AMR) (Prestinaci et *al*., 2015). Nowadays, natural products extracted from plants have become one of the major sources of new drugs. Plants can provide a wide range of complex and structurally diverse compounds. It is well known that plant products constitute a significant sector of the existing antimicrobial compounds (Berdy, 2005).

Many researchers have geared their investigations toward taking advantage of plant and microbial extracts such as essential oils as potential candidates for antimicrobial agents (Runyoro *et al*., 2006; Nazzaro *et al*., 2013). The comparison of the results deduced from different articles on the *in vitro* antimicrobial activities of natural products is of prime importance. However, the comparison is extremely difficult due to the non-standardization of the methodology and the insufficiency of plant products database.

Antibiotics are influential drugs used to fight diseases at large commercial scales. Any powerful antibiotic has a wide range of side effects, consequently, patients taking antibiotics, are subjected to side effects ranging from mild to severe depending on the type of antibiotic, the targeted microbes and the patient himself (Slama *et al*., 2005).

Due to their rich and diverse chemical composition, plants could be very efficient against microbes and thus provide precious sources of natural antimicrobial agents. The elements isolated from plants are important alternatives to many synthetic antimicrobial drugs because of their weak or no side effects and their better bioavailability (Roosita *et al*., 2008). Nevertheless, ensuring minimum toxicity, specific concentrations of the natural products should be considered. Antiquity plants were used as antimicrobial due to their healing and antiseptic properties. *Dittrichia viscosa* (L.) (*D. viscosa)* is a perennial herbaceous plant described as a flowering plant with viscous and glandular leaves (Quezel and Santa, 1963). It is a native of the Mediterranean basin and is

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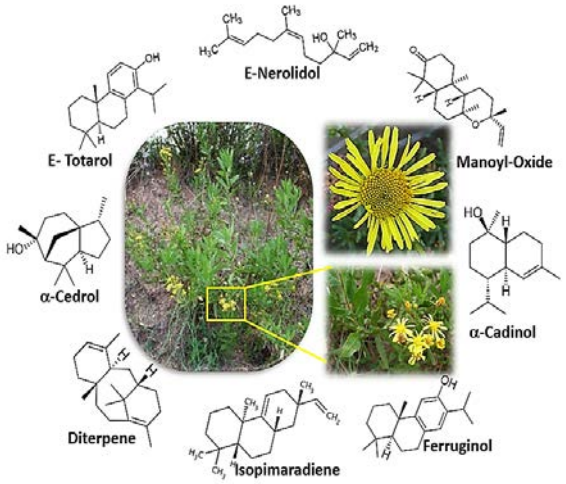
widely distributed in Europe as well as Asia and Australia (Parolin *et al*., 2014). This genus of the *Asteraceae* family includes over 100 species, but only a few species have been tested for their antimicrobial properties (Brullo and Marco, 2000; Seca *et al*., 2014). Figure 1 represents the *D. viscosa* plant, which was reported to possess a high content of phenolic acids and flavonoids. (Grauso *et al*., 2019) interpreted the extensive use of its roots in the traditional Greek-Arabic medicine as an expectorant agent of the mucous membranes to treat cough and catarrh. The medicinal and pharmacological potential of *D. viscosa* plant includes, but not limited to, balsamic, healing, antipyretic, antidiabetic, antichloristic, antiviral, antifungal, antibacterial, and antiseptic applications (Alejandro *et al*., 2008).

Figure 1: Image of D. viscosa and its flowers as well as chemical structure of main volatile constituents.



Historically, the D. viscosa was used to treat wounds and injuries, bruises, and intestinal disorders (Laurentis *et al*., 2002). *D. viscosa* extracts have shown anti-inflammatory activity and free radical-generating characteristics enabling it to be efficient in protecting against enzymatic and non-enzymatic lipid peroxidation (Schinella *et al*., 2002).

The study is focused on characterization and investigating the antimicrobial activities of the oil extracted from leaves and stems of *D. viscosa* plant collected from Bainem forest (northwest part of Algiers). Characterization and the chemical variation of the isolated essential oils (leaves and stems) were compared with results obtained from other regional parts of Algeria as well as other countries such as Italy, France, Portugal, Turkey, Tunisia, Syria, Jordan. Furthermore, the *in vitro* antimicrobial activities of the isolated essential oils (leaves and stem) are conducted using the zone of inhibition (agar disk-diffusion method) (Heatley, 1944; Hudzicki, 2009) to correlate the chemical composition of essential oils and their antimicrobial activities.

2. Materiel and Methods

2.1. Plant material and essential oil preparation

D. viscosa leaves and stems were harvested from the Bainem forest located on the northwest part of Algiers on July 27, 2012. The plant was identified by Higher National Agronomic School (E.N.S.A) in Algeria. Collected leaves and stems samples were left to dry in the dark and at ambient room temperature. Later on, the dried samples were submitted to hydrodistillation using a Clevenger type apparatus for 5 hours as described in the literature (European Pharmacopoeia 6.0., 2008).

2.2. Volatile isolation and identification using GC and GC–MS analysis

The volatile isolated were analyzed by gas chromatography (GC) Hewlett-Packard 6890, equipped with a single injector and two flame ionization detection (FID) systems and two Supelco fused silica capillary columns with different stationary phases. Particularly, SPB-1 (poly dimethyl siloxane of dimensions (30 m × 0.20 mm) and film thickness of 0.20 µm and SupelcoWax-10 (polyethylene glycol) were used. The temperature of the oven was programmed to incrementally increase from 70 to 220 ºC at a heating rate of 3 ºC/min. At a temperature of 220 °C, the system was subjected to isothermal invariance for 15 minutes. It worth mentioning that the temperature of the injector and detectors were fixed at 250 ºC. The carrier gas used was helium, and the splitting ratio was 1:40. The GC-MS was carried out in a Hewlett-Packard 6890 gas chromatograph fitted with an HP1 fused silica column (poly dimethyl siloxane (30 m × 0.25 mm (i.d.)) with the film thickness of 0.25µm). The GC parameters, as described above, were as the following: An interface temperature of 250 ºC, MS source temperature of 230 ºC and the MS quadruple temperature of 150 ºC. The ionization energy and current were 70 eV and 60 µA, respectively.

2.3. Identification

Compounds were identified by their GC retention indices on both SPB-1 and SupelcoWax-10 columns and from their mass spectra. Retention indices calculated by linear interpolation relative to retention times of C8–C23 of n-alkanes (Van den Dool and Kratz, 1963) were compared with those of reference samples included in C.E.F. / Faculty of Pharmacy, University of Coimbra laboratory database. Acquired mass spectra were compared with reference spectra from the laboratory database, Wiley (Wiley, 2007), and validated literature data (Cavaleiro *et al*., 2004; Cavaleiro *et al*., 2011). Relative amounts of individual components were calculated based on GC raw data areas without FID response factor correction.

2.4. Antimicrobial tests

2.4.1. Disc diffusion assay

The essential oils obtained from the aerial parts of *D. viscosa* were tested against four bacteria strains (reference strains) and two yeast strains. The bacteria strains used were *Escherichia coli* (ATCC 4157), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 9372), *Staphylococcus aureus* (ATCC 6538). The two yeasts used were *Saccharomyces cerevisiae* (ATCC 601) and *Candida albicans* (ATCC 24433). The microbial strains were supplied by CRD (Center of research and development) of SAIDAL pharmaceutical group Algiers. To carry out the antibacterial test, the bacteria strains were inoculated into nutrient broth Mueller Hinton (MH) at 37 °C for 24 hours, and then the bacterial suspension was obtainable in the time period of 18-24 hours. The same procedure was

repeated for the yeast strains except the incubation temperature was 30 °C, and the suspension was obtained in a time span of 48 hours. Three to five different bacterial colonies from the same patch were placed individually in 6 mL of sterile physiological water. The focus of 10^6 CFU/ml for wavelength 450 nm was obtained (Hammer and Carson, 1999). The 6 mm diameter paper discs were separately impregnated with 25 µg, 100 µg and 300 µg content of the oil dissolved in dimethyl sulfoxide (DMSO) 10% (v/v) (Sigma Aldrich). The paper discs then placed on the nutrient broth, which had previously been inoculated with the selected test microorganism. The plates were left for 1 hour at 4 °C and then incubated for bacteria at 37 °C for 24 hours in the case of bacteria. For yeast strains, the plates were incubated at 30 °C for 48 hours. In addition, the DMSO solvent that was used to dissolve the extracted oil was also used as a negative control. Standard antibiotics Trimethoprim-sulfamethoxazole, Cefixime, Amoxicillin, and Lymecycline at the concentration of 25 µg/disk were employed as positive controls. Antimicrobial activities were assessed in triplicate based on the inhibition zone radius (z), taking into account the disc diameter (6 mm).

2.4.2. The minimum inhibitory concentration (MIC)

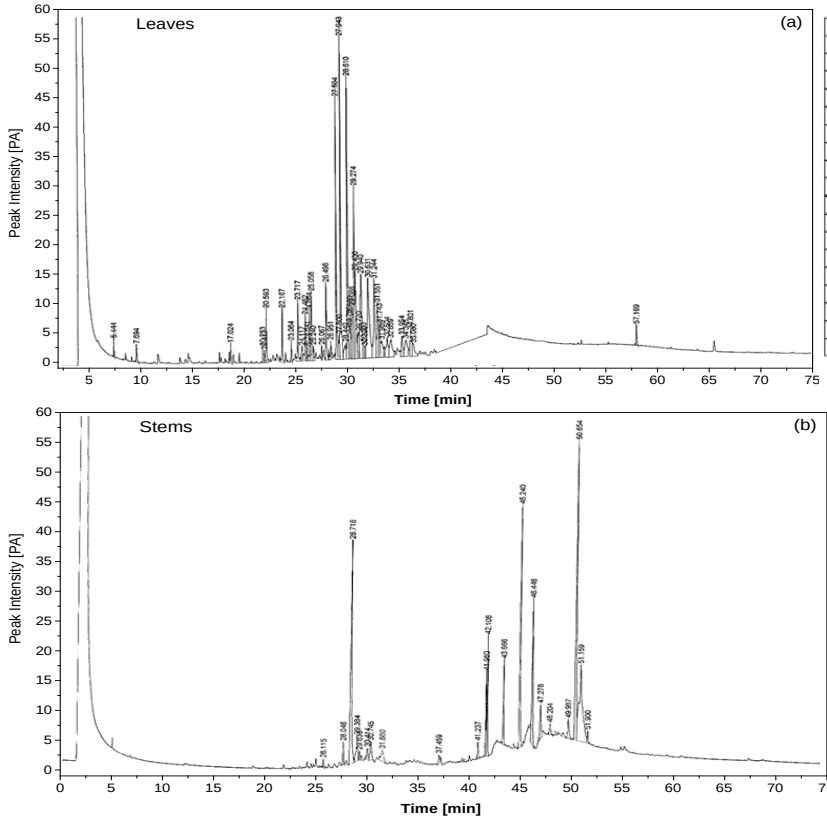
The minimum inhibitory concentration (MIC) of different bacterial samples is determined by diluting the essential oil in Mueller-Hinton-agar (MHA). A bacterial suspension of 10^6 CFU/ml is prepared from a modern bacterial culture from 18 to 24 hours. The serial dilution of essential oils in the MH, ranging from 10 to 300 µg. ml^{-1}, are placed in Petri dishes and exposed to drying, as well as Petri dishes without essential oil. Finally, 1 µl of each microbial strain was added to each Petri dish and placed in an incubator at 37 °C for 24 hours. For each concentration, three tests were carried out.

3. Results and Discussion

3.1. Comparison of the Volatiles Compounds

D. viscosa essential oil was obtained by hydrodistillation of leaves and stems in a yield of 0.15% and 0.036%, respectively. Qualitative and quantitative determinations are given in (Table 1 and Figure 2). The chromatographic profile of stems is characterized by high amounts of chief abundant compounds: diterpenoids (69.1%), cis-totarol (18.1%), α-cedrol (16.7%), ferruginol (16.6%), isoabienol (12.1%), abienol (8.3%), isopimaradiene (5.1%), abietatriene (4.0%) and manoyl-oxide (3.6%).

Investigation of the stems of the species *Inula graveolens* revealed a predominance of oxygenated monoterpenes (60.1%) with bornyl acetate (33.4%) and borneol (21.4%) (Harzallah-skhiri et *al*., 2005). A pioneering study conducted on the stems of *Inula candida* (L.) reported the concentrations of the major components to be the flavonoids composition (0.008-0.023%), phenolic acids (0.411-0.516%), total polyphenols (1.53-1.75%), non-tannin polyphenols (0.56-0.78%) and tannins (0.96-1.06%) (Maleš et *al*., 2010). To the best of our knowledge, the analysis of the chemical composition of the essential oils (stems) derived from Algerian *D. viscosa (*same family of the above examples*)* has not been investigated previously. Hence, the detailed report on the analysis of the chemical composition of the essential oils is of great value for relevant future investigations.



Time	Compound	RI[b]
5.444	α-pinene	929
7.694	limonene	1206
17.024	α-ylangene	1489
20.593	α-copaene	1489
22.167	trans-caryophyllene	1590
23.064	geranyl acetate	1876
23.717	allo-aromadendrene	1638
24.462	ϒ–muurolene	1684
24.641	β-selinene	1716
24.864	selina-3,5-diene	n.d
25.058	Δ-selinene	1716
25.240	α- muurolene	1724
26.067	cis-calamenene	1824
27.504	trans-nerolidol	2032
27.943	caryophyllene oxide	1974
28.610	fokienol	2160
29.940	ϒ-eudesmol	2152
30.631	α-eudesmol	22.09

Time	Compound	RI[b]
26.115	*δ-cadinene*	1747
28.046	*trans-nerolidol*	*2032*
28.715	*α-cedrol*	*2103*
29.384	*T-cadinol*	2152
30.745	*α-cadinol*	2219
41.980	*manoyl-oxide*	2343
42.106	*isopimaradiene*	2287
43.666	*abietatriene*	2469
45.240	*isoabienol*	n.d
46.446	*Abienol*	n.d
49.987	*cis-totarol*	n.d
50.654	*trans-totarol*	n.d
51.159	*Ferruginol*	n.d

Figure 2: Chromatograms and main compounds of the essential oil of **(a)** leaves and **(b)** stems from *D. viscosa* obtained by SPB-1 column.

Essential oils (leaves) are characterized by a high content of oxygenated sesquiterpenes (40.7%), caryophyllene oxide (10.4%), fokienol (9.6%), α-eudesmol (7.6%), trans-nerolidol (7.0%) and γ-eudesmol (6.2 %.) as main components. The chemical composition of leaves is found to differ drastically from that of stems, with co-dominance of trans-nerolidol (7.0%, 1.1%) and caryophyllene oxide (10.4%, 1.1%) for leaves and stems respectively.

Table 1: Chemical composition of the essential oil of *D. viscosa*: leaves and stems.

No.	Compounds	Leaves [%]	Stems (%)	RI[a]	RI[b]
	α-pinene	0.4	-	930	1027
	myrcene	-	t	980	1161
	δ-3-carene	-	t	1005	1152
	limonene	t	t	1020	1206
	terpinolene	-	t	1076	1288
	tepinene-4-ol	-	t	1158	1597
	terpinyl acetate	-	t	1328	1688
	α-ylangene	0.4	-	1364	1489
	α-copaene	1.4	-	1368	1489
	trans-caryophyllene	1.5	t	1406	1590
	geranyl acetate	0.5	-	1429	1876
	allo-aromadendrene	1.8	-	1446	1638
	germacrene D	-	t	1464	1699
	γ–muurolene	1.1	-	1465	1684
	β-selinene	0.6	-	1469	1716
	selina-3,5-diene	1.3	-	1475	n.d
	δ-selinene	2.1	t	1480	1716
	α-muurolene	0.5	t	1484	1724
	cis-calamenene	0.3	-	1506	1824
	δ-cadinene	-	0.4	1507	1747
	trans-nerolidol	7.0	1.1	1545	2032
	caryophyllene oxide	10.4	1.1	1558	1974
	fokienol	9.6	-	1573	2160
	α-cedrol	-	16.7	1576	2103
	γ-eudesmol	6.2	-	1608	2152
	T-cadinol	-	4.9	1615	2152
	α-eudesmol	7.6	-	1627	2209
	α-cadinol	-	1.8	1630	2219
	manoyl oxide	-	3.6	1964	2343
	isopimaradiene	-	5.1	1968	2287
	abietatriene	-	3.6	2018	2469
	isoabienol	-	12.1	2070	n.d
	abienol	-	8.3	2110	n.d
	cis-totarol	-	1.7	2230	n.d
	trans-totarol	-	18.1	2253	n.d
	ferruginol	-	16.6	2271	n.d
GROUPED COMPONENTS					
	Monoterpene hydrocarbons	0.4	0.0	-	-
	Oxygenated monoterpenes	0.0	0.0	-	-
	Sesquiterpene hydrocarbons	11.4	0.4	-	-
	Oxygenated sesquiterpenes	40.7	25.7	-	-
	Diterpenoids	0.0	69.1	-	-
	Geranyl acetate esters	0.5	-	-	-

- Compounds listed in the order their elution from SPB-1 column
- RI[a]: Retention indices in the SPB-1 column.
- Ri[b]: Retention indices in the Supelcowax 10 column.
- t: Traces (<0.05%).

The yields and the main constituents of the *D. viscosa* essential oil extracted from samples of other countries are reported in Table 2.

Table 2: Major compounds of essential oil of *D. viscosa* obtained from different countries and regions as long as the experimental data [(*)].

Country(region) Area	Algeria (north) (Bainem forest) [(*)]	Tunisia (Beja 2015)	Syria Al-Qadmous2014	Spain –Donadio village2000	Italy Sardinian2003	Portugal (Algarve 2008)	Jordan (Irbid 2010)	Turkey (Fethiye1996)	France (Corcia 2006)	Algeria (east) Constantine Hamma Bouziane 2012	Algeria (east) Constantine Ain El-Bey 2012
Data	Experimental	Literature	Literature	Literature	Literature	Literature	Literature	Literature	Literature	Literature	Literature
Yield of essential oil [%]	0.15	t	0.09	-	0.43	<1	0.05	0.20	0.03–0.07	8.0	10.0
Extraction method	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD
Part of the plant	L	L	L	AP	AP	AP	AP	FAP	FAP	FAP	FAP
α-pinene	0.4	-	-	-	-	-	-	0.7	-	-	0.1
α-ylangene	1.4	-	-	-	-	0.5	-	-	0.4	-	-
α-copaene	-	-	-	-	-	0.7	-	-	0.2	-	-
Trans-caryophyllene	1.5	-	-	-	-	-	-	-	0.7	-	-
geranylacetone	0.5	-	-	-	-	-	-	-	0,1	-	-
allo-aromadendrene	1.8	1.3	-	-	-	0.6	-	-	-	-	0.4
γ–muurolene	1.1	-	-	-	-	-	-	-	0.2	-	-
β-selinene	1.3	-	-	-	-	-	-	-	-	-	-
α- selinene	2.1	-	-	-	-	-	-	-	-	-	-
α-muurolene	0.5	-	0.2	-	-	1.3	-	0.1	0.6	-	-
cis-*calamenene*	0.3	-	-	-	-	-	-	-		-	-
trans-*nerolidol*	7.0	-	13.6	7.7	1.9	8.4	19.8	1.5	8.6	9.6	25.3
caryophylleneoxide	10.4	6.7	7.8	0.4	8.0		-	1.5	2.5	0.1	5.5
fokienol	9.6	-	-	38.8	-	-	20.9	-	21.1	7.2	4.4
γ-eudesmol	6.2	-	-	-	-	-	2.6	-	0.1	-	-
α-eudesmol	7.6	-	-	-	-	-	2.7	-	2.2	0.9	-
Oxygenated Sesquiterpenes	40.7	6.7	54.3	-	-	32.7	77.2	-	60.0	-	-

- t: Traces (<0.05%), L: Leaves, AP: Aerial parts,
- FAP: Fresh aerial parts.
- HD: Hydrodistillation technique.

The variations can be attributed to several factors such as the studied part of the plant, collection location, harvest season, and timing of extraction (Miguel et *al.*, 2005; Camacho et *al*., 2003). The major ingredients of essential oils from all regions are oxygenated sesquiterpenes (23.8% to 77.15%).

The obtained results in this work of the chemical compositions of the essential oils (leaves) indicate significant similarities with those extracted from Al-Qadmous of Syria (Nasser et *al*., 2014). Both studies report a predominance of trans-nerolidol (13.64%) with caryophyllene oxide (7.83%). It was reported that the plant extracted from Beja of Tunisia (Alalan et *al*., 2015) contains caryophyllene oxide of 6.67%, slightly smaller than the caryophyllene oxide percentage reported in our work.

Comparing the chemical composition of the aerial parts with the counterparts from samples extracted from Donadio village of Spain (Camacho et *al*., 2003) shows the presence of fokienol (38.8%) and trans-nerolidol (7.71%). The chemical composition analysis of the essential oils extracted from the samples from Irbid in Jordan (Al-Qudah et *al*., 2010) indicates similar compounds with concentrations of fokienol (20.87%), trans-nerolidol (19.75%), α-eudesmol (2.66%) and γ-eudesmol (2.57%). Moreover, plants extracted from Sardinian of Italy (Marongiu et al., 2002) were found to contain 12-carboxyeudesma-3, 11(13)-diene (43.97%), globulol (16.8%), valerianol (12.0%) and caryophyllene oxide (8.0%). Likewise, the plant extracted from Algarve of Portugal (Albano et *al*., 2012) was reported to be consisted of β-oplopenone (7.2%), δ-cadinol (5.5%) and α-cadinol (5.3%).

The analysis of the chemical composition of fresh aerial parts of the plants extracted from the Constantine: Hamma Bouziane and Ain El-Bey eastern part of Algerian (Berhail Boudouda et *al*., 2012) indicate that it contains trans-nerolidol (9.6%, 25.3%), caryophyllene oxide (0.1%, 5.5%) and fokienol (7.2%, 4.4%). Further comparison with samples extracted from Corcia of France (Blanc et *al*., 1996) indicates the presence of trans-nerolidol (8.6%),

caryophyllene oxide (2.5%), and fokienol (21.1%). In addition, the compositional investigations of the fresh aerial parts of essential oils extracted from Turkish Fethiye (Kotan et *al.*, 2007) demonstrate that they contain borneol (25.2%), bornyl acetate (22.5%) and isobornyl acetate (19.5%) as major components.

3.2. Antimicrobial Activities

Essential oils (leaves and stems) exhibit antimicrobial activity that can be basically determined from the size variation of the inhibiting zone. The diameter of the DMSO solvent used as a negative control is 6 mm (diameter of a paper disk). The antimicrobial effect was improved by increasing the essential oil concentration. Concentration of 300 µg is found to exhibit the best antimicrobial activity (Figures 3 and 4).

Remarkably, the essential oil (leaves) showed better inhibitory effect (on bacteria and yeast strains) as compared with oil (stems). This could be interpreted in terms of the existence of a high concentration of the oxygenated sesquiterpenes group (40.7%), as well as the presence of trans-nerolidol (7%). The existence of trans-nerolidol in isolated oil (leaves) is found to play a significant role against *C. albicans* yeast strain only. However, the cis-nerolidol isomer is found to exhibit inhibitory activity against two *C. albicans* strains (Curvelo et *al.*, 2014). The microbial inhibitory role of oil (stems) can be attributed to the existence of diterpenoids (69.1%) (Núñez et *al.*, 2018).

The four bacterial strains were selected deliberately from Gram-positive and Gram-negative strains, to confirm the anticipated fact that Gram-positive bacteria are the most susceptible bacteria to oil extract from *D. viscosa*.

It should also be mentioned that a synergy between the essential oil compounds can contribute to antimicrobial activity (Marino et *al.*, 2001 and Amorim et. *al.*, 2013). (Seca et *al.*, 2013) confirming the importance of the genus *Inula* as a medicines source for humans.

The antimicrobial activities of the essential oil (leaves and stems) were compared with the antibacterial activities of standard antibiotic agents (trimethoprim-sulfamethoxazole, amoxicillin, and lymecycline). The antibacterial efficacy of oil extracted from *D. viscosa* leaves was fair on *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* and almost 40-60 % of the average efficacy of the selected standard antibacterial (Figure 5). Interestingly, at concentration of 25 µg, all antibiotics except Bacillus subtilis show higher inhibition with compare to the essential oil.

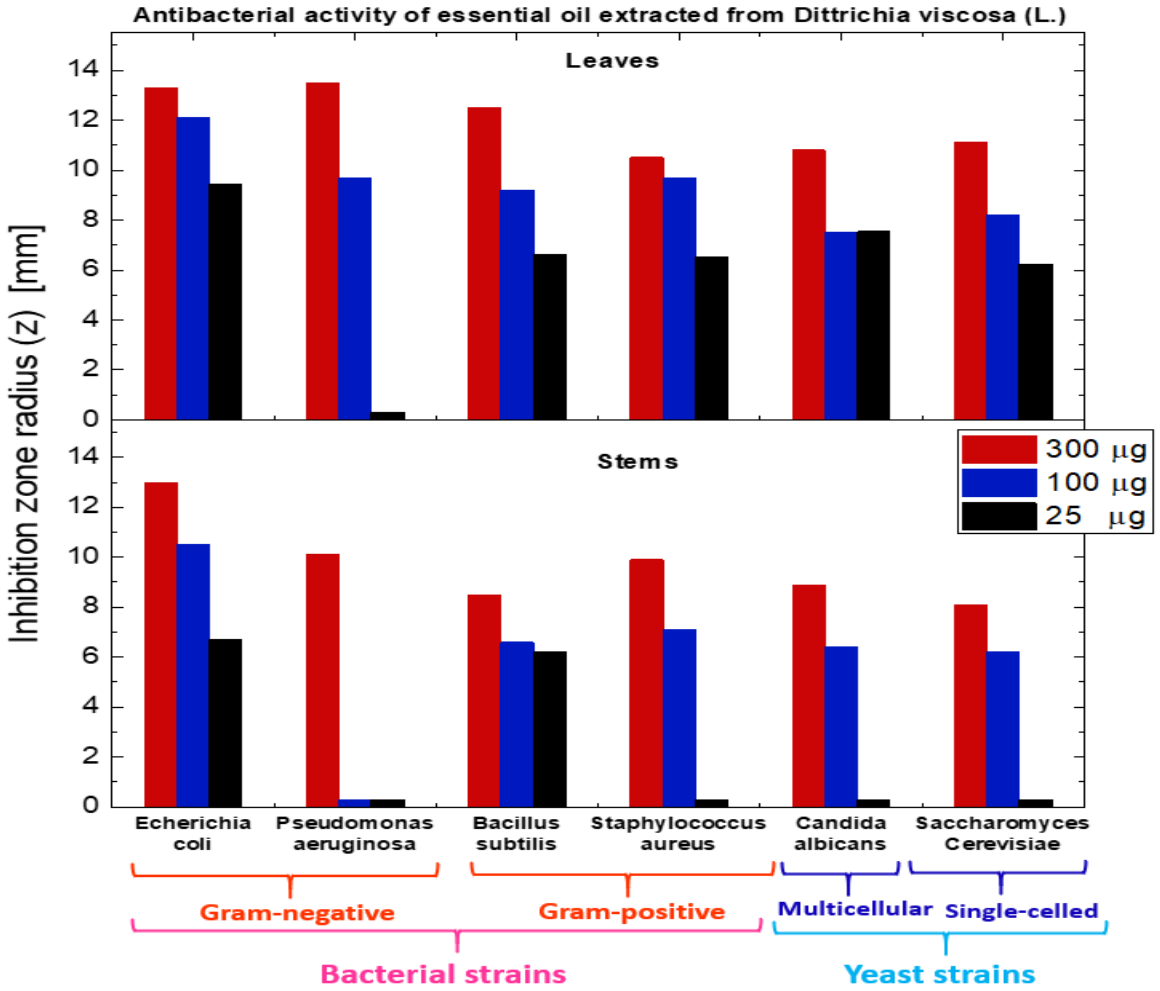


Figure 3: Antimicrobial activities of the essential oil (leaves and stems) of *D. viscosa.* Concentrations of 25 µg, 100 µg and 300 µg were applied on two Gram-negative bacteria strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and other two Gram-positive strains (*Bacillus subtilis* and *Staphylococcus aureus*) as well as two yeast strains (*Saccharomyces cerevisiae* and *Candida albicans*), that are single-celled and multicellular, respectively.

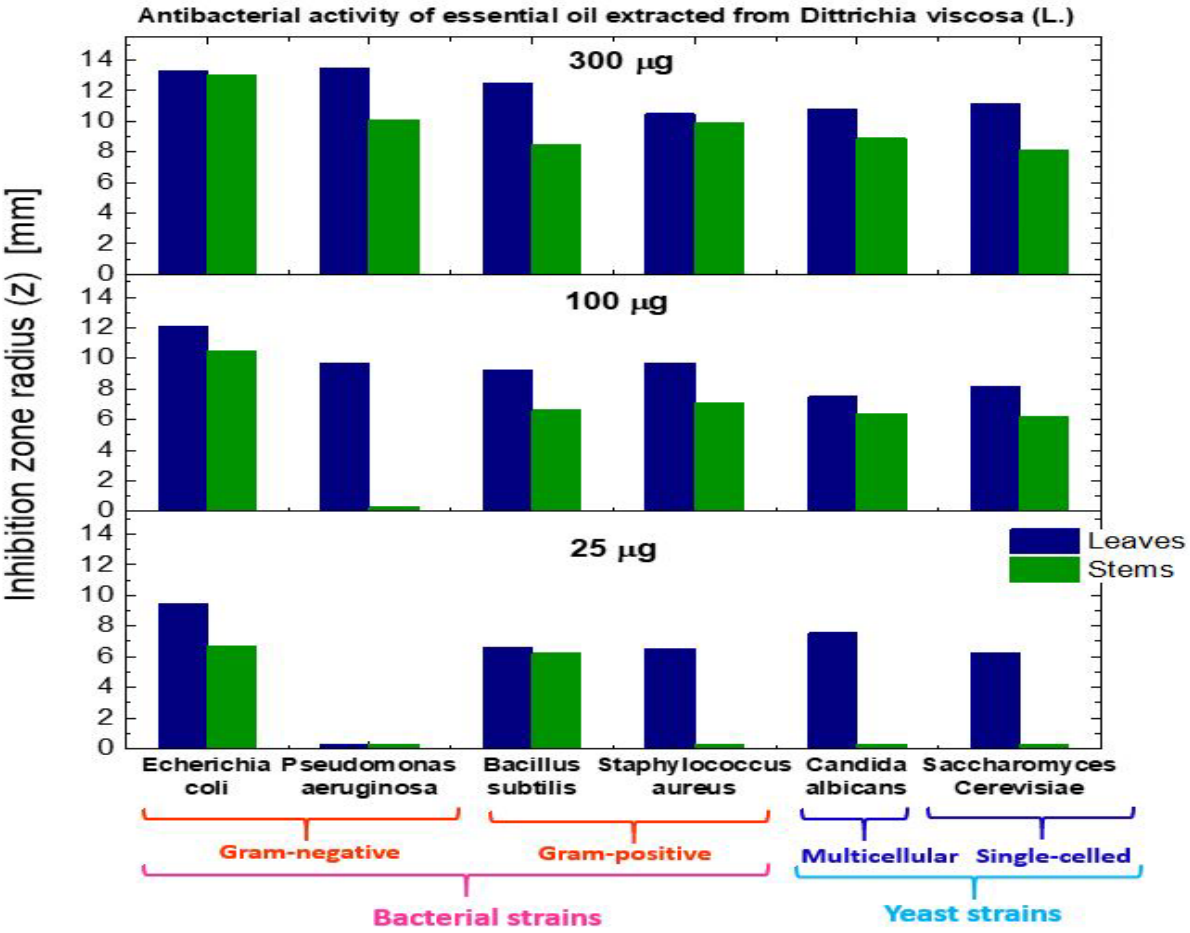


Figure 4: Comparing the concentration effect of isolated essential oil concentration from the leaves and stems of *D. viscosa* on antimicrobial activities. The concentrations are 25 µg, 100 µg and 300 µg applied on two Gram-negative bacteria strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and other two Gram-positive strains (*Bacillus subtilis* and *Staphylococcus aureus*) as well as two yeast strains (*Saccharomyces cerevisiae* and *Candida albicans*), that are single-celled and multicellular, respectively.

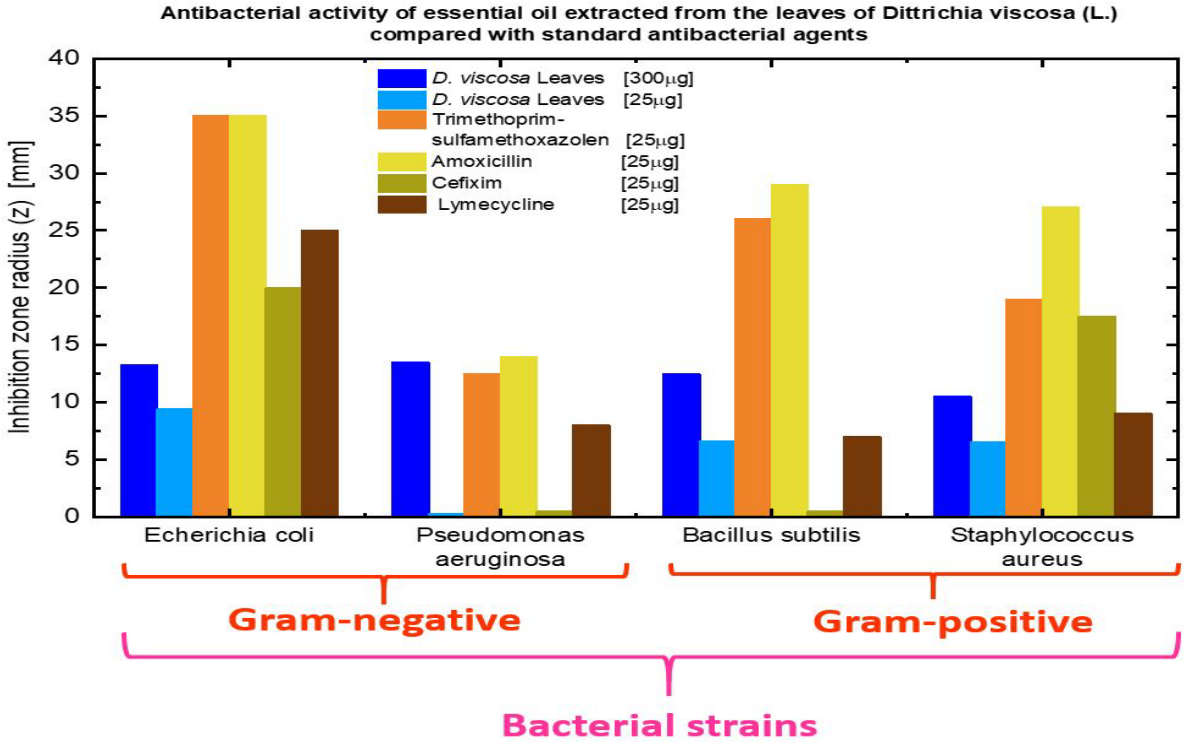


Figure 5: The Antimicrobial activities of isolated essential oil of *D. viscosa* (leaves and stems) at 25 µg concentration as well as standards antibiotics (TRS: Trimethoprim-sulfamethoxazole; CF: cefixime; AMC: Amoxicillin; LE: Lymecycline).

As tabulated in Table 3, the MIC value of the oil on Gram-positive bacteria is 20 µg/ml. However, On Gram-negative bacteria, the MIC values of the oil vary, depending on the strain tested.

Table 3: Minimal inhibitory concentration (MIC) of *D. viscosa* essential oils (leaves and stems).

Minimum Inhibitory Concentration (MIC) [µg.ml^{-1}]		
Microorganism	Leaves	Stems
Gram-negative bacteria		
Echerichia coli	15	20
Pseudomonas aeruginosa	100	300
Gram-positive bacteria		
Bacillus subtilis	20	20
Staphylococcus aureus	20	20
Yeast strains		
Saccharomyces cerevisiae	20	100
Candida albicans	20	100

The MIC values are in the range between 20 and 100 µg/ml. The oil strongly inhibits the growth of *Escherichia coli*. The MIC values indicate that our samples exhibit better antimicrobial activities than that obtained using the oil of leaves of *D. viscosa*, harvested in Tunisia (250 µg/ml) (Gharred et *al.* 2019). A study of antibacterial activity against Staphylococcus aureus ATCC 25923 yields a MIC of 32 µg/ml (Aissa et *al*., 2019). On the contrary, the MIC generated by oil on *Pseudomonas aeruginosa*, is found to be higher and equal to 300 µg/ml and 100 µg/ml for stems and leaves, respectively.

4. Conclusions

In conclusion, the chemical composition of the essential oils extracted from *Dittrichia viscosa* (L.) by hydrodistillation indicates the predominance of the oxygenated sesquiterpenes (40.7 %) in the oil extracted from leaves. The extracted oil from stems is found to contain the diterpenoids (69.1%) substantially. The chemical compositions of the essential oils (leaves and stems) are in good agreement with literature, nonetheless the differences between some results of this work and previous works are mostly due to difference in plant located region, harvest season and timing of extraction.

Essential oils (leaves and stems) exhibit antimicrobial activities which were deduced from the size variation of the inhibiting zone. Furthermore, the antimicrobial activity is found to enhance by increasing the concentration of essential oil. Concentration of 300 µg is found to yield optimum antimicrobial activity.

The oil (leaves) demonstrates a better inhibitory effect against all bacterial and fungal strains compared to oil (stems). This is due to the significant presence of trans-nerolidol (7%), caryophyllene oxide (10.4%) and fokienol (9.6%) in the leaves with compare to stems. Additionally, the trans-nerolidol was observed in isolated oil extracted from leaves only which plays a significant role against *C. albicans* yeast strain.

The antibacterial efficacy of *D. viscosa* leaves oil was found to be fair on *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* and almost 40-60 % of the average efficacy of the selected standard antibacterial.

Despite the fact that trimethoprim-sulfamethoxazole, amoxicillin, and lymecycline are more effective than oil extracted from *D. viscosa* leaves, it is still natural and can be used at higher doses allowed to compensate for the difference inefficiency. However, the toxic doses of the essential oil should be avoided. The essential oil can also be added to standard antibiotics for synergistic therapy since antibiotic combinations are frequently used to treat serious Gram-negative infections.

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