GAS CHROMATOGRAPHY–MASS SPECTROMETRY, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF THE ESSENTIAL OIL OF *ORIGANUM VULGARE* L. SSP. *GLANDULOSUM* (DESF.) IETSWAART AND *THYMUS PALLESCENS* DE NOÉ FROM WESTERN ALGERIA

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Abstract. The chemical composition of the essential oils extracted by hydro distillation of the aerial parts of *Origanum vulgare* L. subsp. *glandulosum* (Desf.) letswaart and *Thymus pallescens* de Noé (Lamiaceae), aromatic medicinal plants endemic to Algeria, was characterized by gas chromatography-mass spectrometry (GC-MS). Hydrodistillation of the aerial parts of *Origanum vulgare* L. subsp. *glandulosum* (Desf.) letswaart and *Thymus pallescens* de Noé gave 2% and 6% oils yield respectively; on a dry weight basis. The analysis of the essential oils allowed us to identify 33 and 43 components, respectively, representing 98.27 and 99.743% of the oil. The major components were carvacrol (36.62%) followed by thymol (27.30%), γ-terpinene (11.15%), p-cymene (5.57%), trans-caryophyllene (3.16%), β-sesquiphellandrene (2.85%) and α-terpinene (1.48%). Overall, these data indicate that *Origanum vulgare* L. subsp. *glandulosum* (Desf.) letswaart could be a source of bioactive compounds. The antioxidant and antimicrobial activities of essential oils extracted via hydrodistillation was also investigated. The essential oils have antioxidant activity, it is directly related to the chemical composition and method used to determine each activity. The antimicrobial test results revealed that the essential oil of *Thymus pallescens* showed the strongest inhibition against the tested microorganisms, with *Staphylococcus aureus* being the most sensitive.

Keywords: *Lamiaceae, chemical composition, Origanum, Thymus, Tissemsilt*

Introduction

Origanum and *Thymus* genera of important medicinal and aromatic plants, belong to the family Lamiaceae. There are about 38 species of the genus *Origanum* widespread in the Mediterranean, Euro-Siberian and Irano-Siberain regions with distinguished odor and flavor of flowers and leaves (Bakha et al.*,* 2019; Cid-Pérez et al.*,* 2016; Kosakowska et al., 2019). It has a great economic importance since it produces secondary metabolites endowed with important functional properties useful in different industrial applications (cosmetics, pharmaceuticals, food) (Zheng et al.*,* 2001). The phenolic content and the

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antioxidant activity of its different subspecies have been widely referenced in the literature (Spiridon etal., 2011; Ličina etal., 2013; Martins et al., 2014). The essential oils and extracts of *Origanum* species are widely used in pharmaceutical, cosmetic and perfume industry, and for flavoring and preservation of several food products. The essential oils of this species are rich sources of carvacrol, a component of particular biological importance: it is known for its antibacterial and antifungal activities, antispasmodic effects, acetylcholine esterase inhibition, lipid peroxidase inhibition, radical scavenging effect, and cardiac depressant activity (Kirimer et al., 1995). Several studies have been published on the biological properties of *Origanum* such as antibacterial (Şahin et al., 2004), antioxidant, anti-inflammatory and anticholinesterase (Loizzo et al., 2015), mutagenic and antimutagenic effects (Mezzouga et al., 2007) and antifungal (Khosravi et al., 2011). It has also been used as a traditional remedy to treat various ailments such as a spasmodic, antimicrobial, and aromatic for whooping and convulsive coughs, digestive disorders and menstrual problems (Sokovic et al., 2002; Daferera et al., 2003).

Origanum vulgare ssp. *glandulosum* (Desf.) Ietswaart, is an endemic spontaneous plant, that grows in North Africa (Algeria and Tunisia) (Ietswaart, 1980). In Algeria, *Origanum vulgare* is an aromatic shrub called Zaatar which is mostly used as a medicinal plant against whooping cough, cough, fever, bronchitis and some gastrointestinal disorders (Baba Aissa, 1991; Aligiannis et al., 2001). It has different positive effects against various pathogens, including fungi, viruses, bacteria and insects (Duschatzky et al., 2005; Pavela, 2009a; 2009b; Tommasi et al., 2023; Polat et al., 2012). In addition, it could represent the right approach for limiting the rising of bacterial resistance to conventional antimicrobial compounds (Marchese et al., 2017; Tu, 2015). The genus *Thymus* comprising around 350 species of perennial aromatic herbs and shrubs predominantly found in the Mediterranean region and Asia (Stojanovic et al., 2008). Eleven *Thymus* is found in Algeria in four sections of this genus (Morales, 2002); *Thymus guyonii* de Noé considered to be rare and endemic to the northern Sahara, *Thymus pallescens* de Noé common and endemic to northern Algeria, and *Thymus numidicus* Poiret, is endemic to the Algerian and Tunisian areas. The *Thymus* genus is widely used as a spice and medicinal herb, with several pharmacological properties such as antispasmodic, antiseptic, antitussive, expectorant, and flatulence-reducing actions. Several species of *Thymus* genus grow in the Algerian wild regions, including rare *Thymus pallescens* de Noé. The *Thymus* genus includes some of the most widely used plants in the world, including herbal teas, flavoring agents, aromatics and medicinal herbs (Stahl-Biskup, 2002, Stahl-Biskup and Stahl-Biskup, 2002). In this study, to our knowledge, we investigated for the first time, the chemical composition of the essential oils of *Origanum vulgare* L. subsp. *glandulosum* (Desf.) letswaart and the essential oils of *Thymus pallescens* de Noé from Bordj Bou Naama region of Tissemsilt city (western Algeria) by GC-MS analysis.

Experimental section

Plant material

Origanum vulgare aerial parts (*Fig. 1*), spontaneously growing in the locality of Bordj Bou Naama, province of Tissemsilt (Northwestern Algeria, at 1408 m above sea level, latitude: 35°51’57.8”N, longitude:1°37’31.8”W), were collected from individuals during the flowering period of June 2021.We have also the *Thymus pallescens* de Noé which growing in the region of Souaad in Tissemsilt (Northwestern Algeria, at 970 m above area level, latitude: 35°50’23.1”N, longitude: 1°36’01.7”W) during the flowering period of June 2021. Botanical determination was performed in the laboratory of *Plant Biodiversity* using available literature (Quezel and Santa, 1962-1963). Before undergoing extraction, plant material was dried in the shadow at room temperature for 7 days.

(a) **(b)**

Figure 1. *Photos of species used (a)Origanum vulgare L ssp. glandulosum (Desf.) letswaart and (b) Thymus pallescens de Noé*

Essential oils extraction

Essential oils were obtained from dried material by hydrodistillation for 3h using a Clevenger-type apparatus. Each essential oil was collected, dried over anhydrous sodium sulphate and stored at 4°C until used.

GC-MS analysis

The essential oils of *Origanum vulgare* and *Thymus pallescens* de Noé were analyzed by gas chromatography spectrometry Hewlett Packard 6890N, driven by an HP5MS capillary column 30 m long, 0.25 mm internal diameter and 0.25µm film thickness. The analyses were performed at isothermal temperatures, with the temperature set to 60°C for 8 min and then increasing by 2°C up to 250°C for 10 min. This operation took 113 min. With an analysis mode, the volume injects 0.2 L at 250°C. Tic scan (30 to 550) and 3.5 min solvent delay at 280°C. An Agilent 5973 mass spectrometer is connected to the gas chromatograph. The flow rate of the helium carrier gas, which has a purity of N6.0 and is managed in split mode 1/50, is 0.5 ml. Ionization was accomplished by electron impact at 70°C and 230°C for the ion source. A homologous series of C7 n-alkanes was used to determine the retention indices (RI) of the constituents using the Kovats method 12 technique. By comparing the constituents’ retention indices to those in the literature as well as their mass spectral fragmentation models to those in the database (Nist library) and the literature, the constituents were identified.

Antioxidant activity

DPPH test

Based on the method of Shirwaikar et al. (2006) for measuring antioxidant activity by DPPH, a DPPH solution (0.1 mm) as prepared in methanol, and then 1.2 ml was added to essential oils at various concentrations. The prepared solutions were left at room temperature for 30 min in the dark before the absorbance was measured with a UV spectrometer at 517 nm. Various concentrations of ascorbic acid (vitamin C) were used as a reference synthetic antioxidant (positive control) and were measured under the same circumstances as the test samples. By calculating the percentage of discoloration (reduction) of DPPH in the solution of methanol, the ability of the antioxidant (extracts or ascorbic acid) to trap the free radical was estimated. The inhibition percentage was calculated using the following equation:

$$\text{Inhibition (\%)} = [A0 - A1/A0] \times 100$$

where A0 was the absorbance of control and A1 was the absorbance of reaction mixture. The value of IC50, the concentration of the required extract to trap 50% free radicals, was calculated by linear regression of calculated inhibition percentages depending on different sample concentrations prepared.

Antibacterial activity

Bacterial strains

The American Culture Collections (ATCC): *Staphylococcus aureus* (ATCC11778), *Pseudomonas aeruginosa* (ATCC10231), *Candida albicans* (ATCC10231), *Escherichia coli* (ATCC25922) *and Bacillus cereus* (ATCC25923) were used in this study to evaluate the antibacterial activity of our two essential oils.

Aromatogram test

The disc diffusion method was used to study the sensitivity of microbial strains to essential oils in comparison to the standard bacterial strains. Mueller Hinton agar (for bacteria) was inoculated with sterile Whatman discs (6 mm in diameter), which were then placed on the central surfaces and impregnated with 100L of essential oils. After the plates; were exposed to bacteria for 24 h at 37°C. The measurements were made of the clear zone's diameters. The antibacterial activity was expressed as the average of the inhibition diameters produced (in mm) for each test. All measurements were performed in triplicate.

Minimum inhibitory concentration by microdilution

The minimal inhibitory concentration (MIC) is the lowest concentration that, after 18 h of culture at 37°C, prevents any discernible growth of a bacterial strain. To determine this MIC, 96-well microplates we used. Briefly, according to the protocol described by Djeussi et al. (2013) with some modifications, 100 µl of Mueller Hinton broth was added to the 96 wells (sterile microplate). Subsequently, 100 µl of dissolved essential oil (50 mg/ml) was added. Then a series dilution was made to obtain a concentration of 25. Thus, 100 µl of the inoculum were added to each well, with the last well used as a negative control. Finally, the samples were incubated at 37°C for 18 h.

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Results and discussion

Chemical composition of essential oils Origanum vulgare L. subsp. glandulosum (Desf.) Ietswaart

Hydrodistillation of the aerial parts of *Origanum vulgare L.* subsp. *glandulosum* (Desf.) Ietswaart gave 2% oils yield on a dry weight basis (w/w) of yellowish good smelling oil. This result is in disagreement with most of the previous works: according to Bekhechi et al. (2008), the average yields of essential oils obtained, from the same plant, vary considerably between the different stations of Beni-Mester (3.53%), Terni (3.90%) and Sebdou (3.95%). The quantitative determination of essential oils in this plant reveals its richness in its secondary metabolites. The phytochemical study of this same species, growing in the region of Setif; carried out by Belhattab et al. (2005), allowed them to obtain essential oils yields of around 2.7%. Ruberto et al. (2002) noted that the essential oils yield of this harvested species in different parts of the region of Setif are variable ranging from 2.3% to 5%. The essential oils yield of this same plant harvested in different stations of the Eastern region of Algeria, carried out by Sari et al. (2006), ranged from 0.8% to 3%. The GC-MS analysis of the essential oil allowed to identify 33 components, representing 98.27% of the oil. The major components were carvacrol (36.62%) followed by thymol (27.30%), γ-terpinene (11.15%), p-cymene (5.57%), Trans-Caryophyllene (3.16%), β-Sesquiphellandrene (2.85%) and α-Terpinene (1.48%) (*Table 1*; *Fig. 2*). This result is in good agreement with other studies on the essential oils of the Algerian *O. glandulosum*, which have shown the almost permanent richness of *Origanum vulgare* essential oils in phenolic monoterpenes, generally carvacrol and occasionally thymol (D'antuono et al., 2000). This composition is similar to those reported in previous studies on *Origanum vulgare* L. subsp. *glandulosum*, which showed the presence of 4 major compounds such as thymol, carvacrol, p-cymene and γ-terpinene with the majority of samples belonging to chemotypes, carvacrol and thymol (Khalfi et al., 2008). The predominance of carvacrol recorded was described in a recent study on samples of the same species collected in the Bougaa region of Sétif with a percentage of 26.29% (Ali et al., 2020). Considering the endemic character of this plant in North Africa, a study in the Mediterranean Phyto-region of North-East Tunisia, showed that carvacrol with a value ranging from 61.08% to 83.37% was also the main compound of this *Origanum* species (Béjaoui et al., 2013). On the other hand, according to Nabti et al. (2020), carvacrol was present in a small percentage (2.8%) with thymol as chemotype (56.3%) in the chemical composition of the oil extracted. However, these authors (Nabti et al., 2020) found a dominance of carvacrol (59.6%) in the Bordj-Bou Arreridj region for the oil of the species studied. According to Belhattab et al. (2005), the phytochemical study of *Origanum glandulosum* from the Sétif region in Algeria shows that the essential oils of this plant have high levels of carvacrol (47%), followed by g-terpinene (13.4%), p-cymene (11.2%) and thymol (6.6%). In addition, the analyzes chromatography performed by Ruberto et al. (2002) on *Origanum glandulosum* essential oils, harvested from four different stations in the region of Setif, reported the presence of two chemotypes: thymol and carvacrol chemotype, followed by p-cyme (3.6-25.8%) and g-terpinene (4.8-13.2%). Sari et al. (2006) carried out a study of the chemical variability of the essential oils of *Origanum glandulosum* collected from various regions of Eastern Algeria. They put forward the existence of two chemotypes: chemotype thymol and carvacrol chemotype. The obtained results showed that the identified majority compounds are thymol (18.5-73.1%), carvacrol (7.6-72.6%), p-cymene (1.7-18.5%) and g-terpinene (1.1-18.7%). These results highlight the great variability of the chemical composition of *Origanum vulgare* L. subsp. *glandulosum* (Desf.) letswaart essential oils through their components. The variation in oil yield and oil constituents may be attributed to different environmental factors taking into account that oil is a metabolic product of plant cells and its quantitative and qualitative composition may be influenced by the species, soil, climate conditions, altitude, drying methods, seasonal variation, plant organ, vegetative cycle stage, harvesting time, geographical origin, temperature and extraction technique.

Chemical composition of essential oil Thymus pallescens de Noé

The essential oils were extracted by a hydrodistillation process using a Clevenger-type apparatus. The extracted oils were dried over anhydrous sodium sulphate, and stored at 4°C. The essential oil yield of the aerial part of *Thymus pallescens* de Noé was 6% in the oil on a dry basis (w/w). GC/MS analysis allowed to identify 43 components, representing 99.27% of the oil (*Table 1; Fig. 3*). According to Chikhoune (2007), the yield of this plant was about 4.6%, which is much lower than our yield. In the other regions of Algeria, the production of essential oils in *Thymus* samples from Kadiria (Bouira) was relatively average (3. 3%).The essential oil of the *Thymus pallescens* of our study was characterized by the presence of 4 majority compounds: carvacrol, the -terpinene, p. cymene and α-terpinene with concentrations of 60.91%, 15.61%, 7.61%, 2.66% respectively, which agrees with previous studies with only slight differences:

Moutassem et al. (2021) revealed that phenols were the most important portion of the essential oils of *Thymus pallescens*. The major constituents were carvacrol (56.64%) and p-cymene (16.36%), followed by thymol (8.71%), γ-terpinene (5.4%), α-pinene (3.33%), and linalool (3.12%). According to Benchebane (2015), Oxygenated monoterpenes (74.3–78.7%) constituted the main fraction of the oils, with carvacrol (63.3–68.2%) being the most prominent oil component followed by p-cymene (6.2–10.3%), thymol (6.5–7.5%) and γ-terpinene (6.9–7.4%). According to Hazzit et al. (2013) and Hazzit and Baaliouamer (2009), oxygen containing monoterpenes were the main group of constituents and the predominant components were carvacrol, γ-terpinene and p-cymene. According to our species phytochemistry, our results appear to be characterized by the dominance of a phenolic component, carvacrol, with a content of 60.91%, followed by monoterpenic hydrocarbons. γ-Terpinene (15.61%), p-Cymene (7.61%) and α-terpinene (2.66%). According to the analysis of Hazzit (2007) using GC and GC/MS, carvacrol (39.7–65.0%) was found to be the most abundant constituent at each harvest period, followed by p-cymene (5.1–15.1%) and γ-terpinene (6.1–17.4%).

The chromatographic analysis performed by Alouane (2015) showed no qualitative change between the controls and irradiated samples. Oils were characterized by high carvacrol content (81.8-85.7%) followed by linalool (3.7-5.0%). These results disagree with those reported by Hazzit (2007), Chikhoune (2007), Hazzit et al. (2009) and Benchabane et al. (2015). The extraction method quality and quantity can vary depending on environmental conditions, plant organ, vegetative cycle stage, and harvesting time (Abu Lafi et al., 2008; Wang et al., 2012).

Table 1. *Chemical composition of Origanum vulgare L. Subsp. glandulosum (Desf)Ietswaart and Thymus pallescens de Noé aerial parts essential oils by GC-MS analysis*

N°	RT	KI	Compound	*Origanum vulgare*	*Thymus pallescens*
1	3.74	****	Methyl 2-methylbutyrate	0.0644	0.045
2	8.66	925	α-Thujene	0.3929	1.4
3	9.1	932	α-Pinene	0.1994	1.676
4	10.07	948	Camphene	0.0245	0.102
5	11.49	972	Sabinene	*	0.044
6	11.91	979	β-Pinene	0.0548	0.253
7	12.31	986	(3E)-Octen-2-ol	0.1299	0.072
8	12.64	992	β-Myrcene	0.9998	1.829
9	13.7	1008	α-Phellandrene	0.222	0.402
10	14.49	1019	α-Terpinene	1.4814	2.665
11	15.2	1029	Para-cymene	9.5732	7.619
12	15.37	1031	D-Limonene	0.227	0.629
13	15.48	1033	B-Phellandrene	0.2526	0.403
14	16.63	1049	(E) –β-Ocimene	0.0423	0.23
15	17.74	1064	y-Terpinene	11.1503	15.619
16	18.52	1075	Cis-Sabinene hydrate	0.1527	0.2
17	19.32	1087	Terpinolene	0.0523	0.104
18	20.81	1107	Linalool	0.7009	1.523
19	21.1	1111	Trans-Sabinene hydrate	*	0.054
20	25.95	1178	Santolinyl acetate	0.087	0.086
21	26.5	1186	Ternin-4-ol	0.5106	0.244
22	26.8	1190	α-Terpineol	*	0.051
23	28.38	1212	Iso-dihydrocarveol	0.0309	*
24	29.6	1229	(3Z) Hexenyl 2-methyl butanoate	*	0.054
25	30.55	1243	Carvacrol, methyl ether	0.1171	0.107
26	35.02	1307	Thymol	27.3031	0.13
27	35.5	1315	Carvacrol	36.6242	60.912
28	39.44	1375	α-Copaene	*	0.098
29	39.78	1380	2.5-Di-tert-butylhydroquinone	*	*
30	40.19	1386	Geranyl acetate	*	0.294
31	41.38	1404	Longifolene	*	0.476
32	42.26	1418	Trans-Caryophyllene	3.1631	0.884
33	43.1	1432	Trans-α-Bergamotene	0.0934	*
34	43.42	1437	Aromadendrene	*	0.185
35	44.52	1454	α-Humulene	0.2677	0.034
36	44.8	1459	Alloaromadendrene	*	0.083
37	45.76	1474	γ –Muurolene	0.0576	0.14
38	46.12	1480	Germacene D	0.0424	0.114
39	46.3	1483	Nerylisobutanoate	0.0578	*
40	46.67	1489	cis-β-Gualene	*	0.237
41	47	1494	Bicyclogermacrene	*	0.132
42	47.24	1498	α-Muurolene	*	0.029
43	47.87	1508	β-Bisabolene	0.5228	*
44	48.2	1514	γ-Cadinene	*	0.132
45	48.4	1517	δ-Cadinene	*	0.25
46	48.93	1526	β-Sesquiphellandrene	2.8532	*
47	49.81	1541	(E)-α-Bisabolene	0.2321	*
48	52.1	1580	Spathulenol	*	0.098
49	52.27	1583	Caryophyllene oxide	0.5955	0.079
50	55.87	1646	α-Muurolol	*	0.025
Total				**98.2789**	**99.743**

(RT): retention time, (KI): Kovats index, (*): below of detection limit

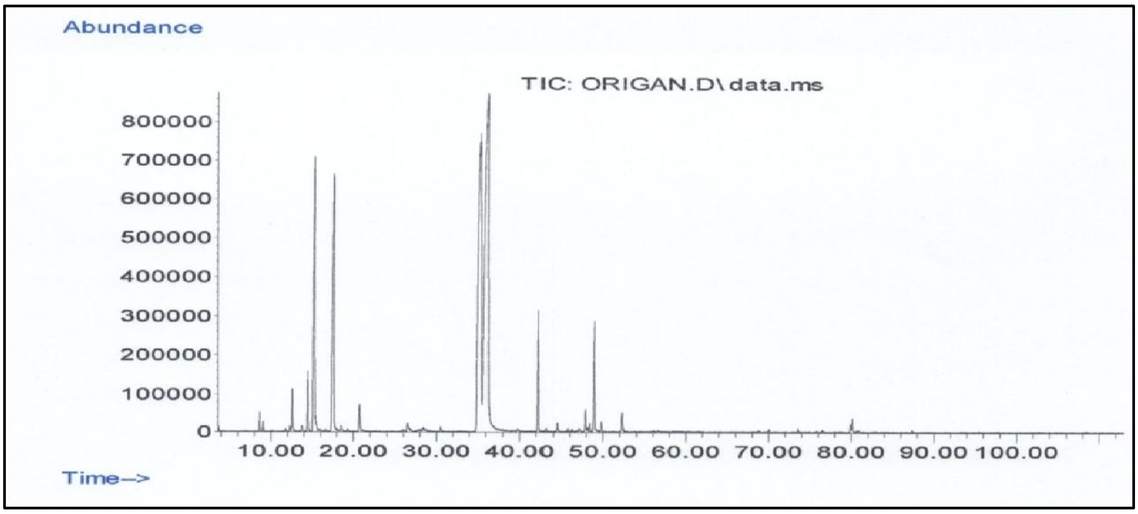


Figure 2. *GC–MS chromatogram of Origanum vulgare L. Subsp. glandulosum* (Desf) Ietswaart *essential oil (abundance as function the retention time in min)*

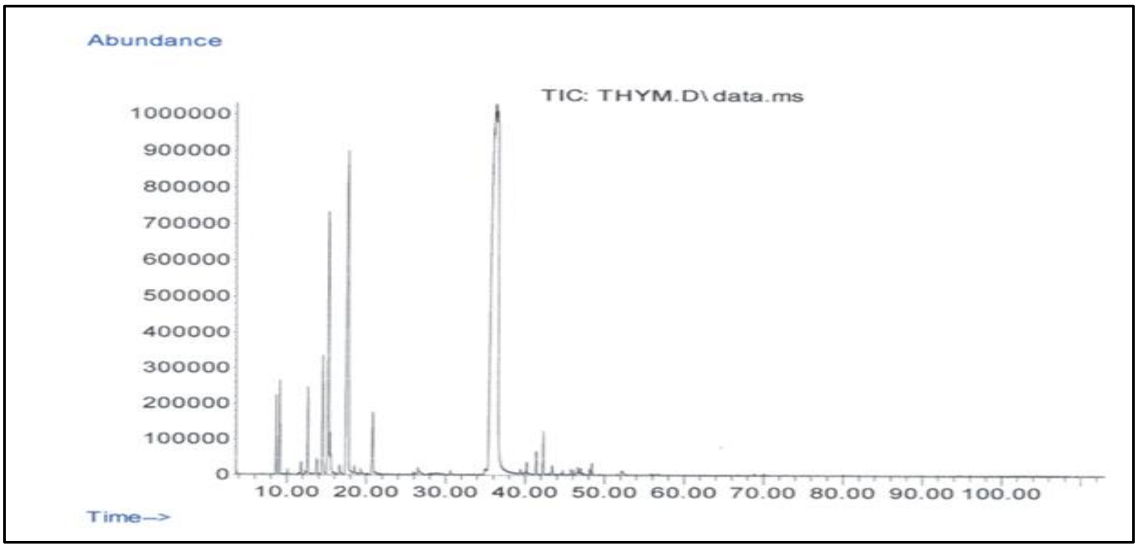
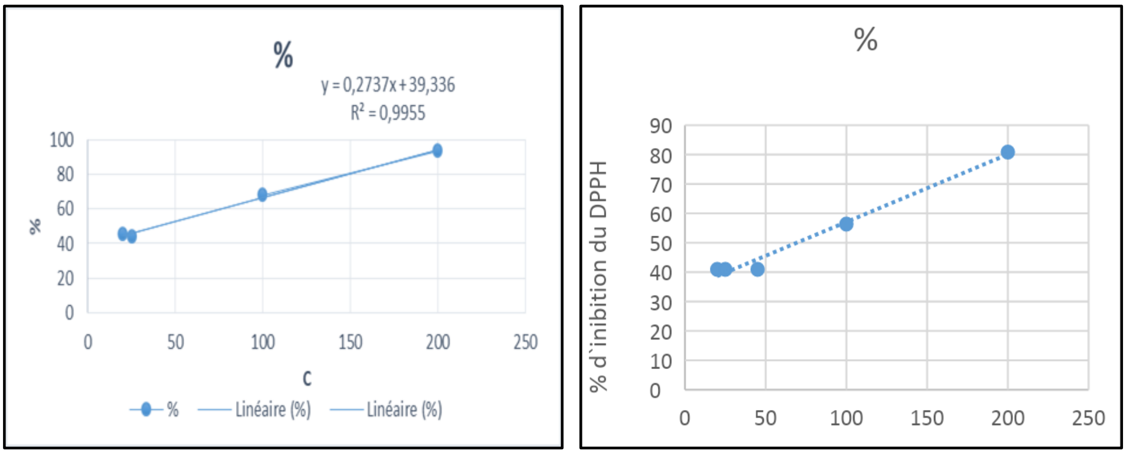


Figure 3. *GC–MS chromatogram of Thymus pallescens de Noé essential oil (abundance as function the retention time in min)*

Determination of antioxidant activity

The results of the DPPH method showed good activity for the essential oils *Origanum vulgare* and *Thymus pallescens* de Noé obtained by the hydrodistillation process. In addition, our oils are more active because they contain a major component in their chemical formulation which are carvacrol and thymol for *Origanum vulgare* and carvacrole for *Thymus pallescens* de Noé. In addition, the presence of other compounds like γ -terpinene, p-cymene and α-terpinene appear to be important and necessary to promote antiradicalar testing. The activities of recovery of radicals DPPH essential oils of *Origanum* and *Thymus* in methanol exist, because they are able to reduce the stable DPPH radical which is violet color to yellowish diphenylpicrylhydrazine with IC50 values varying from 38.96 and 69.27 µg/ml respectively. The antioxidant activity of

Origanum vulgare essential oils and *Thymus pallescens* de Noé evaluated by the DPPH method yielded values that allowed us to plot curves of the percentage of antiradical activity for both oils (*Fig. 4*). From the results shown in *Figures 2* and *3*, we can see that the antioxidant activity is dose-dependent because it is proportional to the increase in the concentration of our samples. The percentages of antiradical activity allowed us to determine the IC50, which corresponds to the concentration of essential oils, necessary for the inhibition of 50% of the DPPH present in our solution. Note that the lower the IC50 the greater the antioxidant activity of the compound. Results are expressed as IC50 values. A study conducted by Sari et al. (2006) on several populations of *Origanum* from different Algerian areas (Sétif, Bejaia, Biskra, Msila and Bordj Bou Arreridj) found good anti-glare capacity of essential oils that gave IC50 values ranging from 16.2 to 26.7. However, Mechergui et al. (2010) showedthat essential oils of *Origanum* from two Tunisian regions has a good antioxidant activity with IC50 of 105.29 mg/L and 142.86 mg/L. In addition, Béjaoui et al. (2013) who conducted a study on the same essential oils species from Tunisia found an IC50 of 625 µg/ml, implying higher antioxidant activity. Based on the comparison with our results, it was determined that the antioxidant activity of our essential oils is related to its chemical composition and major compounds. In contradiction with the work of Gachkar et al. (2007) where they demonstrated that oils with monoterpenic predominance have a fairly modest activity. This implies that the antioxidant activity does not only depend on the chemical composition of our plant but also on the experimental methods used.



(a) **(b)**

Figure 4. *Results of the DPPH test of the two species:(Origanum vulgare and Thymus pallescens de Noé) (a) essential oils of Thymus pallescens de Noé (b) essential oils of Origanum vulgare*

Determination of antimicrobial activity

The results of the method of discs and microdilution of the essential oils of *Origanum vulgare* and *Thymus pallescens*, allowed us to obtain the results presented in *Tables 2, 3* and *4*. *Origanum vulgare* showed a good antibacterial effect on all bacterial strains which reveals that its strains are very sensitive to our essential oils. In addition, the essential oil of *Thymus pallescens* de Noé in turn exhibited an excellent effect on all bacterial strains, but in the case of extracts it was found that they have an effect on all strains and especially for the yeast strain of *Candida albicans* except for *Pseudomonas* and *E. coli* (*Table 2*).

Table 2. *Antimicrobial activity of Origanum vulgare and Thymus pallescens de Noé*

	Origanum vulgare **D (mm)**		***Thymus pallescens*** **D (mm)**	
Strains	**Essential oils**	**Extracts**	**Essential oils**	**Extracts**
Escherichia coli	100%	23mm	100%	00mm
Staphylococcus aureus	100%	35mm	100%	19mm
Bacillus cereus	100%	8mm	100%	16mm
Pseudomonas aeruginosa	100%	19mm	100%	00mm
Candida albicans	100%	32mm	100%	40mm

Based on the results grouped in *Table 2*, we find that our *Origanum* extract has a moderate inhibitory activity towards *Pseudomonas aeruginosa* (19 mm), a strong inhibitory activity against Staphylococcus *aureus* (35 mm), *Bacillus subtilis* (8 mm) and *Escherichia coli* (23 mm), while a strong inhibition was recorded (32 mm) for *Candida albicans*. In the case of *Thymus pallescens* de Noé*,* no inhibitory activity was found against *Pseudomonas aeruginosa* (00 mm) and *Escherichia coli* (00 mm), a moderate inhibitory activity against *Staphylococcus aureus* (19 mm), *Bacillus subtilis* (16 mm), while a strong inhibition was noted with the highest value recorded (40 mm) against *Candida albicans.* It is evident that some strains are resistant against our extracts, such as *Pseudomonas* and *Escherichia coli* and others are either sensitive or very sensitive as in the cases of *Bacillus, Staphylococcus* and *Candida albicans.* Among the studies that have determined the antimicrobial power of essential oils of certain species of *Origanum vulgare*, Bekhechi et al. (2008) tested *Origanum vulgare* essential oils on few bacterial strains, namely *Escherichia coli, Pseudomonas* and *Staphylococcus* that occupied inhibitory zones of 17.6mm, 08mm and 25.6mm, respectively. According to Bijaoui et al. (2013), the antibacterial activity of essential oils. Of *Origanum vulgare* ssp.*glandulosum* against microorganisms, tested in our study, was evaluated by assessing the presence of inhibition zones and MIC values. The highest activity was observed against *Escherichia coli* with the largest inhibition zones (18, 22 and 23 mm) recorded for the late, early and flowering stages, and *S. typhimurium* with large inhibition zones (15, 22, and 22.5 mm). However, *Pseudomonas aeruginosa* was resistant to the studied essential oils (<15 mm of inhibition zones). According to Allouane (2007) the diameters of the inhibition zones obtained were between 27.0 and 52.8 mm. These results show strong activity against all bacterial strains tested, with the exception of *Pseudomonas aeruginosa*. The highest sensitivities were observed against yeast strains (*Candida albicans* and *Saccharomyces cerivisea*). The evaluation of the antibacterial activity of the five OEMs against six uropathogenic MDRs and two standard *Escherichia coli* strains revealed that the five essential oils were active against all strains tested, showing similar inhibition zone diameters and MIC and MBC values (Nabti et al., 2020). Our *Thymus pallescens* samples may contain a high reservoir of phenolic monoterpenes (carvacrol) which has been highly studied for its inhibitory and disintegrating properties on the external membrane of Gram-negative bacteria (La Storia et al., 2011). In addition, there are several authors who found that carvacrol joined the amine and hydroxylamine groups of bacterial membrane proteins, altering their permeability and causing bacterial mortality (Lamert et al., 2001; Juven et al*.,* 1994). The strains (*Staphylococcus aureus* and *Bacillus subtilis*) are more sensitive to essential oils than gram-negative bacteria (*Escherichia coli*). The resistance of gram-negative bacteria lies in the presence of essential oils components related to the complexity of the external membrane of microorganisms, containing LPS lipopolysaccharides that prevent the accumulation of the essential oils on cell membrane (Helander et al., 1998).

Determination of minimum inhibitory concentration (MIC)

The inhibitory power of essential oils has been confirmed by tests to determine MIC. *Tables 3* and *4* show the minimum inhibitory concentrations of *Origanum vulgare* and *Thymus pallescens* de Noé essential oils obtained by the direct contact method in an agar medium. MICs are inversely proportional to the diameters of the inhibition zones, obtained with the antibioaromatogram method. The minimum inhibitory concentrations in µg/ml of the essential oils of *Origanum vulgare* and *Thymus pallescens* obtained by the direct contact method are shown in (*Tables 3* and 4). Where they were strongly correlated with the diameters of the inhibition zones. Based on sensitivity, the lowest MIC (50 µg/ml) was obtained for *Origanum vulgare* essential oils on *Pseudomonas aeruginosa* strains: ATCC10231, *Bacillus cereus:* ATCC25923, *Escherichia coli* and *Candida albicans*. In the case of *Thymus pallescens* essential oils, the MIC value for the strain *Pseudomonas aeruginosa* was found to be extremely resistant. Therefore, our results reveal that our plant oils collected from the Bordj bou Naama region has shown good inhibitory activity on gram-positive bacteria like *Staphylococcus* as compared to the gram-negative bacteria *Pseudomonas* ATCC11778. However, it was weakly active on the *Candida albicans* strain. The most sensitive bacteria to the essential oils studied were Gram-positive bacteria in the case of *Thymus pallescens* oils and Gram-negative bacteria in the case of *Origanum vulgare* oils. According to the inhibition zones and the MIC values generated by the essential oils studied, *Thymus pallescens* de Noé essential oils has the best activity on all strains tested. Of the two oils tested, antimicrobial activities have been recorded on all strains are in variation in agreement and disagreement with those reported by previous work for the *Thymus vulgaris* species (Abdeli, 2018) in which he showed that susceptibility was particularly high in *Bacillus cereus* to Tlemcen *Thymus* oils and in *Escherichia coli* and *Pseudomonas mirabilis* to Mostaganem *Thymus* oils. The MIC was recorded at the lowest value, which is in the order of 0.312 µl/ml. In contrast, sensitivity is much lower in *Pseudomonas aeruginosa*; the MIC was obtained at higher values, from 1.25 to 5 µl/ml. Moreover, Bekhechi et al. (2018) found the essential oils of *Origanum glandulosum* very active on all strains tested, except *Pseudomonas*, *Klebserae* and L*isterias*, which were very resistant similarly, Bendahou et al. (2008) found that *Pseudomenas aeruginosa* (P1) and *Pseudomenas aeruginosa* (P2) appeared to resist *Origanum glandulosum* oils at 110.00 lg/ml. The maximum activity was observed against fungi as bacteria, such as *Candida albicans* (Ca) 444, *Candida herbarum* (Cla) 3369 and *A. flavus* (Asp) 994224 with MIC values of 56.00–56.50 lg/ml, 56.50–50.25 lg/ml and 55.25–52.25 lg/ml with HD and SFME oils, respectively. We have, on the other hand, found the bacterial strain of *staphylococcus* was strictly resistant. Essential oils containing large amounts of phenolic monoterpenes, such as carvacrol and thymol, are widely recognized for their excellent antimicrobial properties (Chouhan et al., 2017). These compounds are able to align between fatty acid chains to form a kind of channel through the membrane and are also able to interact with transmembrane proteins, thus affecting the permeability of microbial cells. In addition, they are can disintegrate the outer membrane of Gram-negative bacteria by releasing lipopolysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane (Hyldgaard et al., 2012).

Table 3. *Minimum inhibitory concentration (MIC) of Thymus pallescens de Noé*

Strains	50	25	12.5	6.25	3.12	1.56	0.78	0.3	0.1	0	T +	T-
Escherichia coli	-	CMI	+	+	+	+	+	+	+	+	+	-
Staphylococcus aureus	-	-	-	-	CMI	+	+	+	+	+	+	-
Bacillus cereus	-	CMI	+	+	+	+	+	+	+	+	+	-
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+	+	-
Candida albicans	CMI	+	+	+	+	+	+	+	+	+	+	-

The inhibitory power of essential oils was confirmed by MIC tests. T + with oil and T- without oil

Table 4. *Minimum inhibitory concentration (MIC) of Origanum vulgare*

Strains	50	25	12.5	6.25	3.12	1.56	0.78	0.3	0.1	0	T +	T-
Escherichia coli	CMI	+	+	+	+	+	+	+	+	+	+	-
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	-
Bacillus cereus	CMI	+	+	+	+	+	+	+	+	+	+	-
Pseudomonas aeruginosa	CMI	+	+	+	+	+	+	+	+	+	+	-
Candida albicans	CMI	+	+	+	+	+	+	+	+	+	+	-

The inhibitory power of essential oils was confirmed by MIC tests. T + with oil and T- without oil

Conclusion

The essential oils of the Algerian *Origanum vulgare* L. subsp. *glandulosum* (Desf.) letswaart and *Thymus pallescens* de Noé from Bordj Bou Naama, Tissemsilt, were characterized based on their chemical compositions. The former mainly form of carvacrol, thymol, γ-terpinene, p-cymene, Trans-Caryophyllene, β-Sesquiphellandrene and α-Terpinene while the latter is distinguished by contents ofcarvacrol, γ-Terpinene, p-Cymene and α-terpinene. These two genera are distinguished by a wide range of morphological and chemical diversities consistent with the ways in which they have traditionally been used to treat different infections. This is because they generate secondary metabolites with significant functional properties useful in a variety of industrial application fields (cosmetics, drugs, food). The two studied plants effectively were proving to fight off five different microbial strains, including *Bacillus cereus*, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, and *Candida albicans*. Thymol and carvacrol are evidently the cause of the biological activities of the essential oils of *Thymus pallescens* de Noé and *Origanum vulgare*, respectively. This implies that these phenolic compounds work in synergy with other elements of oils. Therefore, further research into the biological properties of these plants, including their insecticide and antifungal effects, would allow.

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