Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2021, Volume 12, Issue 6, Page 746-756

http://www.jmaterenvironsci.com

Biological activity and characterization of essential oil of areal part from *Origanum majorana* L.: First report of antifungal activity against *Fusarium oxysporum* and against his biofilm

Kaouther Raouafi[1,2], Houwaida Nefzi[1,2,3], Badiaa Esghaier[4], Najla Sadfi[4], Manef Abderrabba[1], Sameh Ayadi[2*]

[1]*Laboratory of Materials, Molecules and Applications, IPEST, Preparatory Institute of Scientific and Technical Studies of Tunis, University of Carthage, Sidi Bou Said road, B.P.512070, La Marsa, Tunisia.*
[2] *Chemistry Department, El Manar University, Faculty of Sciences of Tunis, B.P: 248, El Manar II, 2092 Tunis, Tunisia.*
[3]*Biorefinery Processes Research Group, Department of Chemical and Environmental Engineering, University of the Basque Country (UPV/EHU), Plaza. Europa 1, 20018 Donostia-San Sebastian, Spain.*
[4]*Laboratory of Mycology Pthology and Biomarkes, Faculty of Science of Tunis.*
[5]*Institut National de Recherche et d'Analyse physico-chimique (INRAP). Technopole, 2020, Ariana-Tunisia.*

Received 04 April 2021,
Revised 24 May 2021,
Accepted 27 May 2021

Keywords
- ✓ *Origanum majorna* L,
- ✓ *Antifungal activity,*
- ✓ Antibacterial activity,
- ✓ Antibiofilm,
- ✓ Chemical composition

* *sameh_ayadi2003@yahoo.fr*

Abstract

Origanum majorana L. is an aromatic plant belongs to the family of flowering plants *Lamiaceae* (L).It considered one of the most important temperate culinary herbs. For this, his essential oil has a good economic and industrial importance. This study is focusing on the valorization of the essential oil (EO) of the Tunisian *Origanum majorana* L. For this purpose, the characterization by GC/MS, antibacterial, antifungal and antibiofilm activities of the EO were determined. The results showed that, the major compounds of the EO was terpinen-4-ol(26.7%), γ-terpinene(16.96%), p-menthenol(11.85%), α-terpinen(9.22%), α-terpineol(5.76%) and p-cymene(5.27%).Moreover, this EO presents a good biological activity, it is able to inhibit gram positive and gram negative of bacteria strain, besides, it has an excellent antifungal activity by inhibition of *Fusariumoxysporm* with 100 % and an important inhibition of *Candida albicans* with 43.5 %.

1. Introduction

Essential oils are avolatile compound composed by complexe mixture present at low concentration, this compounds are extracted from different parts of aromatic plantsusing various techniques such as hydrodistillation or solvent extraction [1]. They are widely important in phytosanitary control, enabling the development of other techniques to decrease the negative effects of oxidants, radicals and microorganisms, causing losses to food industries [2]. In addition, essential oils extracted from aromatic and medicinal plants are a source of bioactive compounds, they known by their biological activity in vitro; include antioxidant, antibacterial, antifungal, anti-inflammatory, a good stimulan, reduce nervous headache, antispasmodic and more other effects [3-4].*Origanum majorana* L. is annual, medicinal and aromatic plants species belongs to the family of flowering plants Lamiacea (L), he was known as one of the most used plant in the word due to his biological importance and its diversity of chemical composition [5]. It is a perennial plant originally native to southerm Europe and the Mediterranean

region; however, it is cultivated in various countries [6-9]. This plant is very used in the Mediterranean diet, holding known anti-inflammatory, antibacterial, antifungal and antioxidant properties [10-15]. In other hand, various researchers confirm that the essential oil of *Origanum majorana* L. species is a source of active compounds with great biological importance; It is recognized by a wide range of therapeutic properties such as antispasmodic effects, lipid peroxidase inhibition, acetylcholineesterase inhibition, cardiac depressant activity and radical scavenging effect. Essential oil of *Origanum majorana* L. are caracterized by dominance of monoterpenes where terpinen-4-ol is the most abondant compound in marjoram oil [16-17].
The present study aimed to characterize the chemical composition, antibacterial, antifungal and antibiofilm activities of essential oil extracted from areal part of *Origanum majorana* L.

Abbreviations: Essential oil (EO), minimal inhibition concentration (MIC), minimal bactericidal concentration (MBC), minimal fungicidal concentration (MFC), Diméthylsulfoxide (DMSO).

2. Material and Methods

2.1. Plant material

Origanum majorana L. used in this work was harvested through a biological culture in Nabeul (Tunisia) in May 2017. The fresh aerial material was dried at room temperature, and then it was milled, and finally stored in a closed container before use.

Figure 1: Photo of *Origanum majorana* L.

2.2. Chemicals

The Reagents used in this work were:
Ethyl acetate (99.98%, Fisher chemical Scientific UK, Loughborough),Dimethyl sulfoxide (99.9%, PanReac AppliChem, Germany), Methanol (99.99%, Fisher chemical Scientific UK, Loughborough).

2.3. Extraction of essential Oil

300g of fresh aerial part of plant material was hydrodistilled using a Clevenger apparatus for 3h. The collected EO washed using anhydrous sodium sulfate and then conserved in the dark at 4°C for further analysis [18].

2.4. Chemical analysis of essential oil (GC / MS)

In order to know the chemical composition of the collected EO, it was performed by GC/MS. For this, 10µL of essential oil were dissolved in ethyl acetate. Then, A GC coupled with an Agilent 5975C mass spectrometry detector was used to analyze the solution was analyzed by GC–MS technique. In addition, ionization voltage of mass spectrometer in the EI-mode wasto70 eV 166, however ionization source temperature was 250 °C. The operating conditions are: split in let mode (10:1), carrier gas N2 at a flow rate 0.7 mL/min; injection at 280 °C. Identification of oil is by matching themass spectral fragmentation patterns of different compounds with data from the Mass Spectral Libraryusing the mass finder 3 [18].

2.5. Antimicrobial activity

2.5.1. Agar diffusion method

In present work, a list of Tunisian clinical pathogen strains was carried out: Three gram-negative bacteria: *Klebsiella pneumonia (K.pneumonia), Escherchia coli (E.coli)* and *Enterobacter cloacae* (*E.cloacae*), one gram-positive bacteria: *Staphylococcus aureus (S.aureus),* one yeast strain *Candida albicans (C.albicans)* also one phytopathogen fungi belonging to *Fusarium oxysporum (F.oxysporum)*. To assess the antibacterial activity, EO was diluted in DMSO to 10^{-2} to obtain a concentration of 9.25 mg/mL, sterilized by filtration through a 0.2 µm pore size filter before detection antimicrobial activity.In this study, antibacterial and antifungal tests were carried out by agar well diffusion according to the method described by Dharajiya et al. [19]. Broth microdilution assay using sterile Mueller–Hinton media (BioRad, France) for bacterial strains and yeast malt extract agar YMA (Bio-Rad, France) for antifungal tests were used. A freshly cell suspension (0.1mL) adjusted to 10^7 CFU/mL for bacteria and 10^5 spores mL^{-1} for fungus were inoculated onto the surface of agar plates. Afterwards, wells with 6 mm diameter were punched in the inoculated agar medium and 30 µL of the essential oil were added to each well. Negative controls consisted of using 30 µL DMSO. The plate was allowed to standfor 40 min at 4°C to permit the extract diffusion followed by incubation at 37°C for 24 h for bacteria and at 48h for yeast and the incubation was taken at 30°C for 3-4days for *Fusarium oxysporum*. The antibacterial and antifungal activity was evaluated by measuring the zones of inhibition (clear zone around the well) against the test micro-organisms. All tests were repeated three times.

2.5.2. Determination of Minimum Inhibitory Concentration (MIC), Minimal bactericidal concentration (MBC) and Minimal fungicidal concentration (MFC)

The minimum inhibitory concentration (MIC) of the essential oil was determined using the microdilution broth method. MIC was estimated visually (absence of turbidity) and was determined with three independent measurements. Minimal bactericidal and fungicidal concentrations (MBC and MFC) were determined from the microdilution plates used in the MIC assay. Aliquots (10µL) of each well without visible growth were transferred to plates containing the corresponding media culture, and then incubated at 37°C for 24 h then colony growth was verified. All assays were performed in triplicate according to khemiri et al. [20].

2.5.3. Antibiofilm activity

In order to evaluate the antibiofilm activity of EO, we applicated some method described by Matei et al. [21]. Thus, tubes containing 100 mL of PDB were introduced with a culture of *Fusarium oxysporum* and a volume of 100 µL of diluted EO (10^{-1}) with a concentration of 92.5 mg mL^{-1} and then incubated for 14 days for biofilm formation. The percentage of inhibition of growth rate was measured using the following equation:

$$\frac{R1 - R2}{R1} X100$$

Where: R1: dry weight of control and R2: dry weight of fungal in the presence of compound. Triplicate measurements were realized.

3. Results and discussion

3.1. Yield and GC-MS analysis of essential oil

The dried aerial part yielded 1.72% of EO was analyzed by GC–MS. Thus, the determination of sample composition (%) was based on the peak area normalization with any use of correction factors. Our results showed that the composition of EO contain 26 compounds representing 99.73% of the total oil. According to their retention, the percentage of compounds is listed in Table 1. Our study shows the dominance of oxygenated monoterpenes and monoterpene hydrocarbons fractions ranging from (48.19%-46.92%) with small quantities of sesquiterpene hydrocarbons (1.63%) and oxygenated sesquiterpenes (0.7%) in the oil. This dominance was represented by the presence of the major compounds: terpinen-4-ol (26.7%), γ-terpinene (16.96%), p-menthenol (11.85%), α-terpinen (9.22%), α-terpineol (5.76%) and p-cymene (5.27%).

Table 1: Chemical composition of EO of *Origanum majorana* L.

N°	RT	Components	Area (%)
1	5.80	Trans-thujene	1.9
2	5.65	α-pinene	0.74
3	6.31	β-phellandrene	3.82
4	6.39	β-pinene	0.36
5	6.55	β-myrcene	1.35
6	6.84	α-phellandrene	0.51
7	7.06	**α-terpinen**	**9.22**
8	7.21	**p-cymene**	**5.27**
9	7.30	α-thujene	3.72
10	7.83	**γ-terpinene**	**16.96**
11	7.99	β-terpineol	2.67
12	8.37	Cis-carene	3.07
13	8.99	**p-menthenol**	**11.85**
14	9.95	Borneol	0.24
15	10.22	**Terpinen-4-ol**	**26.7**
16	10.49	**α-terpineol**	**5.76**
17	10.88	isopiperitone	0.94
18	12.11	Anthranilicacid,linalyl ester	1.42
19	12.23	Methylcycooctanol	0.28
20	12.70	3-hexyne-2,5-dimethyl	0.21
21	13.27	Bornylacetate	0.24
22	16.40	Geranylacetate	0.14
23	17.51	Caryophyllene	1.27
24	19.28	γ-elemene	0.36
25	20.92	Spathulenol	0.7
Total			99.73 %
Hydrocarbon monoterpenes			46.92 %
Oxygenated monoterpenes			48.1%
Hydrocarbon sesquiterpenes			1.63%
Oxygenated sesquiterpenes			0.7 %
Others			2.29 %

RT : Retention Time

These results are in accordance with previous studies reporting that the fractions of oxygenated monoterpenes and monoterpene hydrocarbons for *Origanum majorana* constitute the major fractions (56.1%, 39.4%) whose terpinene-4-ol (28.96%), cis sabinene hydrate(17.5%), γ-terpinene (10.5%), p-cymene(9%), α-terpineol(5.6%) and α-terpinene (4.7%) are the major compounds [22]. Other study showed that terpinen4-ol, p-cymene and γ-terpinene (34,4%, 7% and 6.89%) represented the major components of *Origanum majorana* [23]. Other works reported that for *Origanum majorana* the presence of terpinen-4-ol is characterized as major compound [24-25]. Several studies reported that the difference in essential oil composition from Origanum dependents in the difference of climate, soil, nutrients present in the soil and condition of extracting [26].

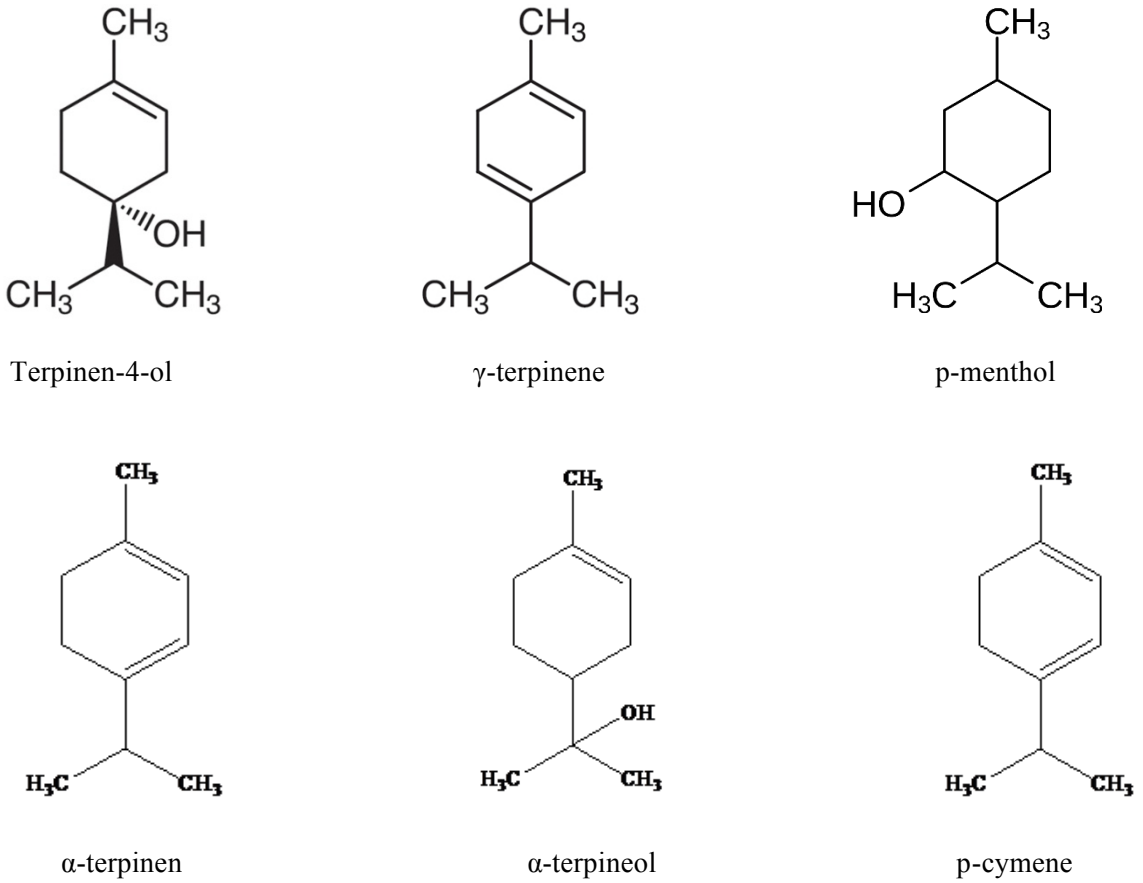


Figure 2: Molecular Structure of the major compounds

3.2. Biological activity

3.2.1. Antibacterial activity

The antibacterial activity of essential oil of *Origanum majorana* L. against four bacterial strains is summarized in Figure 3 and Table 2. This activity expresses in terms of inhibition diameter and by determination of MIC and MBC of EO. The results revealed that EO of *Origanum majorana* L. has an important bactericidal effect to inhibit growth of all tested Gram positive bacteria and Gram negative bacteria with a zone of inhibition varied from 20 ± 0.7 mm to 39 ± 1.41 mm. Compared with the standard agent (ceftaxidime CAZ30), the inhibition diameter of *S.aureus* (39 ± 1.41 mm) and *k.clocae* (39 mm ± 0.8) are higher than ceftaxidime CAZ30 (12 ± 0.3 mm and 23 ± 0.4 mm). Moreover the inhibition diameter of the other strain (*K.pneumonia* and *E.coli*) is close to the standard with values of 20 ± 1.7 and 20 ± 1.2. Thus, the data confirmed the important inhibitory power of this EO, which depends on the

tested strain. The values of MIC and MBC are represented in Table 2, the MIC values varied from 9.25 to 92.5mg mL^{-1} and MBC from 0.925 to 9.25 mg mL^{-1}. The weak value of MIC and MBC was observed against *E.cloacae* and against *E.coli* which confirm the good activity of EO against these two clinical pathogen strains. In general all tested bacteria are sensitive to essential oil of *Origanum majorana* L. however *E.cloacae* and *E.coli* are the most sensitive. According to the literature, our results are in agreement with other works. Olfa et al. [27] demonstrated that Gram negative bacteria are more sensitive than Gram positive bacteria to the EO of *O. majorana* L.

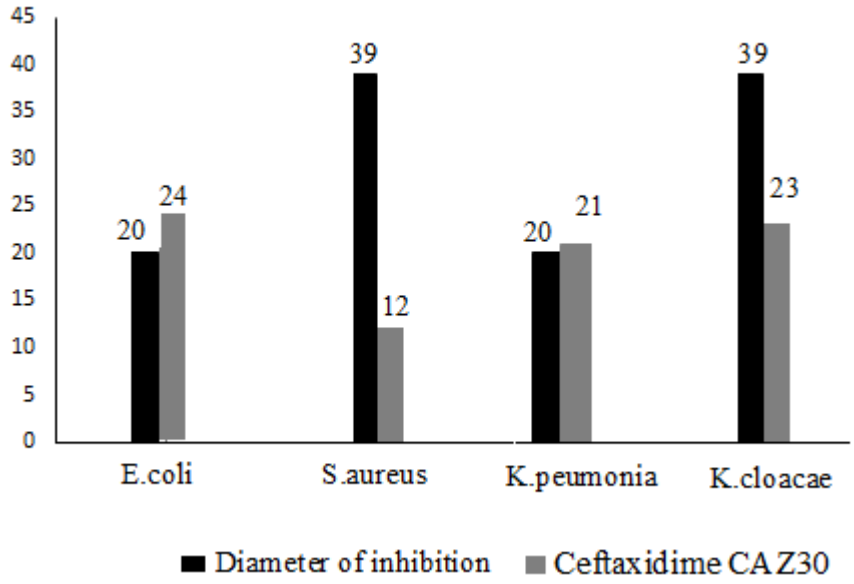


Figure 3: Zone of inhibition of essential oil of *Origanum majorana* L. against bacterial stain expressed in mm.

Table 2: Values of MIC and MBC of EO of *Origanum Majorana* L.

Tested strain	MIC (mg mL^{-1})	MBC (mg mL^{-1})
E.coli	9.25	0.925
S.aureus	92.5	9.25
k.pneumonia	92.5	9.25
E.cloacae	9.25	0.925

Likewise 10 microorganisms were tested; the results indicated that under the action of essential oil of *Origanum majorana* L. all strains were inhibited and thestrain of Gram negative are more sensitive than Gram positive with a MIC ranging from 0.069 to 2.3 mg / mL [28].Another study reported that of all strains both Gram positive and Gram negative were inhibited by the essential oil of *Origanum majorana* with a zone of inhibition ranging from 12 mm to 16 mm [29]. Indeed, multiple studies have reported the significant antibacterial power of marjoram oil and its high capacity to inhibit the growth of Gram + and Gram – strains [30-31]. Other work has proven also that antibacterial agents have an important bactericidal activity for Gram positive more than Gram negative [22, 32-33]. Difference in the antibacterial activities is related to difference of membrane structure; the double membrane structure of Gam negative strains makes them more resistance than strains of Gram positive which characterized by single membrane structure, as well as the concentration and the nature of the active compounds of EO as well the difference in rate of penetration of the constituents into the cell membrane [27]. However, in our present study, properties of antibacterial activity of EO can be associated to the major monoterpenes

compounds such as terpinen-4-ol, γ-terpinene, α-terpinen, α-terpineol and p-menthenol, p-cymene also the synergy between the various majority and minority compounds is taken into consideration. This hypothesis is an agreement with Hajlaoui et al. [22] who confirmed that the antimicrobial activity of marjoram oil is linked to its content of oxygenated monoterpenes (α-terpineol, terpinen-4-ol, α-pinene and p-cymene) and other compounds such as β-Caryophyllene and γ-terpinene. Also, Baydar at al. [34] mentioned that the existence of p-cymene and γ-terpinene is the origin of the antibacterial activity of some plants of Lamiacea (*Origanum minutiflorum,Origanum onites, Thymbra spicata* and *Saturejacuneifolia*).

3.2.2. Antifungal and antibiofilm activity

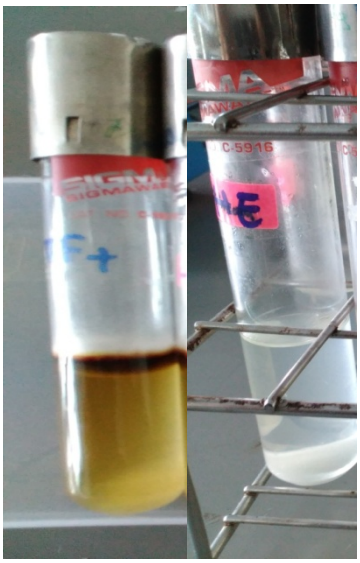
Antifungal activity of EO of *Origanum majorana* L. were evaluated against the phytopathogen *Fusarium oxysporum* and one yeast strain *Candida albicans* as compared to the standards antifungal agent Voriconazole (VCZ), results are listed in Table 3. Our study showed that essential oil of *Origanum majorana* was able to inhibit the growth of *Fusarium oxysporum* and *Candida albicans,* the inhibitory power varies according the tested microorganism. It was very active against *Fusarium oxysporum* species with 100% of inhibition; it has a good antifungal effect against mycelium growth and it has completely inhibited the spore germination, also it reduced the growth of *Candida albicans* with a diameter of 43.5±2.12 mm (43.5 %) with the MIC and MFC values of 0.925 mg mL^{-1} and 9.25 mg mL^{-1}. For *Fusarium oxysporum*, the MIC and MFC are 9.25 $10^{-4}$and 9.25 10^{-3} respectively.

Table 3: Antifungal activity of EO of *Origanum majorana* L. and Values of MIC and MFC

Tested strain	Percent of inhibition (%)	MIC (mg/mL)	MFC (mg/mL)	Zone of inhibition of Voriconazole (VCZ)
Fusarium oxysporum	100	9.25 10^{-4}	9.25 10^{-3}	38 ± 0.5
Candida albicans	43.5	0.925	9.25	30 ± 1.3

In this work, we discussed the anti-*Fusarium oxysporum* effect of EO of areal part of *Origanum majorana L*, In other researchers, Dhaouadi et al. [35] evaluated the antifungal activity of EO from other organ of *Origanum majoranaL*(stem, collar, root and leaf) against eleven isolate of *F.oxysporumf.sp.melonis* and ten isolate of *F.solani*, he mentioned that growth inhibition of EO of roots was 100 % while the growth of inhibition for the rest ranged between 44.84 and 84.13 %. For the *F.solani*,the inhibitory power of all organ of *Origanum majorana* L. varied from 33 to 74.92 %. In the same study, EOfrom organ from *Lavender* has a weak effect against *F.oxysporum* (52.2 -89.90 %) and against *F.solani* (35.90-83.51 %). Other work determined the effect of EO from some Lamiacea species like *C. umbrosa*, *N. leucophylla*, *N. ciliaris* and *N. clarkei* against *F.oxysporum* at 500 µg mL^{-1}, he demonstrated that these plants are able to inhibit the growth of *Fusarium oxysporum* at percent equal to 67.4, 71, 74.5 and 68 % [36].The growth of *Candida albicans* was moderately reduced by essential oil of *Origanum majorana*: the zone of growth inhibition was 11.33 ± 0.57 mm while the MIC and MFC was 0.468 and 1.875 mg mL^{-1} [22]. In addition, it was showed that EO of *Origanum majorana* failed to inhibit the growth of *Candida albicans* [37]. Antifungal activity of EO can be associated to the major compounds of monoterpens or by the synergetic effect of compounds [22]. Inouye et al. [38] showed that concentration of oxygenated compounds influences directly in the antifungal effect. Another author like Ghada et al. [18] showed that terpinen-4-ol was the primary active ingredient for the antifungal tests. In conclusion, antifungal activity of essential oil depending on the composition and the concentration of EO [39].

Due to the high activity of EO of *Organum marjorna* L. against *Fusarium oxysporum*, the activity against biofilm of this phytopathogen was determined. The Results showed that the EO presents hasa great antibiofilm activity; it was inhibited 100% (Figure 4). The formation of biofilm from fungi plays an important role; it can increase the resistance of antifungal compounds. The results are illustrated in Figure 4. According to the Figure 4, essential oil of *Origanum majorana* L. has a good inhibition against biofilm of *Fusarium oxysporum:* the percentage of inhibition was 100% using 100µL compared to untreated biofilm. Works on antibiofilm activity of *Fusarium oxysporum* are very limited. Referring to the literature EO of some medicinal plants such *Thyme* and *clove* inhibited totally the biofilm formation of *Fusarium oxysporum*at *50* µL [39].



1 **2**

Figure 4: Effect of EO of *Origanum majorana* L. against biofilm formation of *F.oxysporum*
With; 1: untreated biofilm; 2: response of biofilm in presence of EO

In particular EO of numerous plants such *oregano*, *rosemary*, *thyme*, *cloven lippies pp*, *cypress* and *citrus* showed a good potential as antibiofilm agent [39]. In our report, the antibiofilm activity is probably related to the richness of essential oil of *Origanum majorana* L. in monoterpens. In another report, Ben Abdallah et al. [40] proved that that the significant antibiofim activity of *Origanum majorana* L. is caused by its high content of Terpinen-4-ol. It has been noted that, the good antibiofilm activity of selected plant was attributed by terpenes [39] which confirms our results found.

Conclusions

In this work, EO has been extracted from Tunisian *Organum marjorna L*. Then, the chemical composition has been determined using GC-MS method. The major components of the essential oil of *Organum marjorna L* were terpinen-4-ol (26.7%), γ-terpinene (16.96%), p-menthenol (11.85%), α-terpinen (9.22%), α-terpineol (5.76%) and p-cymene (5.27%). Moreover, the results confirm that this EO has a good biological activity; we have shown on the one hand the major inhibitory power of essential oil of *Organum marjorna* L. against gram positive and gramnegative bacterial strains, on the other hand against the phytopathogen '*Fusarium oxysporum'* and the yeast '*Candida albicans.* Further study was needed to evaluate the potential use of the essential oil of *Organum marjorna* L. in biological applications as antifungal agent.

References

1. M. E. Lucchesi, F. Chemat, J. Smadja, Solvent-free microwave extraction of essential oil from aromatic herbcomparison with conventional hydro-distillation, *Journal of Chromatography*, 1043 (2004) 323-327.
2. M. Pateiro, F.J. Barba, R. Dominguez, A.S. Sant'Ana, A.M. Khaneghah, M. Gavahian, J.M. Lorenzo, Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review, *Food Research International,* 113 (2018) 156-166.
3. A.C.U. Lourens, D. Reddy, K. H. C. Başer, A.M. Viljoen, S.F. Van Vuuren, In vitro biological activity and essential oil composition of four indigenous South African Helichrysum species, *Journal of ethnophamacology*, 95 (2004) 253-258
4. A.C. Goren, G. Topçu, G. Bilsel, M. Bilsel, Z. Aydogmus, J.M. Pezzuto, The chemical constituents and biological activity of essential oil of *Lavandula stoechas ssp*. Stoechas, *Zeitschriftfür Naturforschung C*, 57 (2002) 797-800.
5. W.H. Talib, I.A. Al-Ataby, A.I. Mahmod, S. Jawarneh, L.T. Al Kury, I.H. Al-Yasari, The Impact of Herbal Infusion Consumption on Oxidative Stress and Cancer: The Good, the Bad, the Misunderstood. *Molecules,* 25 (2020) 4207.
6. F. Sahin, M. Gulluce, M. Daferera, D. Sokmen, A. Sokmen, M. Polissiou, G. Agar, H. Ozer, H, Biological activities of the essential oils and methanol extract of *Origanum vulgare ssp*. vulgare in the Eastern Anatolia region of Turkey, *Food control*, 15(2004) 549-557.
7. C. Busatta, A.J. Mossi, M.R.A. Rodrigues, R.L. Cansian, J.V.D. Oliveira, Evaluation of *Origanum vulgare*, essential oil as antimicrobial agent in sausage, *Brazilian Journal of Microbiology*, 38(2007)610-616.
8. C. Busatta, J. Barbosa, R.I. Cardoso, N. Paroul, M. Rodrigues, D.D. Oliveira, R.L. Cansian, Chemical profiles of essential oils of marjoram (*Origanum majorana*) and *oregano* (*Origanum vulgare*) obtained by hydrodistillation and supercritical CO_2, *Journal of Essential Oil Research*, 29(2017) 367-374.
9. A. Carrasc, E. Perez, A.B. Cutillas, R.M. Gutierrez, V. Tomas, J. Tudela, *Origanum vulgare* and *Thym* bra capitata essential oils from Spain: determination of aromatic profile and bioactivities, *Natural product communications,* 11 (2016) 113-12
10. J.L. Markes, L.M. Volcao, G.D. Funk, I.S. Kroning, W.P. Da Silva, A.M. Fiorintini, Antimicrobial activity of essential oils of *Origanum vulgare* L. and *Origanum majorana* L. against Staphylococcus aureus isolated from poultry meat, *Industrial Crops and Products,* 77 (2015)440-450.
11. T. Baj, A. Baryluk, E. Sieniawska, Application of mixture design for optimum antioxidant activity of mixture of essential ois from Ocimum basilicum L, *Origanum majorana* L and *Rosmarinus Officinalis* L, *Industrial Crops and Products*, 115 (2018) 52-61.
12. V.M. Thanh, L.M. Bui, L.G. Bach, N.T. Nguyen, H.L. Thi, T.T. Hoang Thi, *Origanum majorana* L. Essential Oil-Associated Polymeric Nano Dendrimer for Antifungal Activity against Phytophthora infestans, *Materials,* 12 (2019) 1446.
13. A.K. Chaudhari, V.K. Singh, S. Das, J. Prasad, A.K. Dwivedy, N.K. Dubey, Improvement of In vitro and in situ antifungal, AFB1 inhibitory and antioxidant activity of *Origanum majorana* L. essential oil through nano emulsion and recommending as novel food preservative, *Food and Chemica Toxicology,* 143 (2020)111536.

14. C. Busatta, R.S. Vidal, A.S. Popiolski, A.J. Mossi, C. Dariva, M.R.A. Rodrigues, R.L, Cansian, Application of *Origanum majorana* L. essential oil as an antimicrobial agent in sausage, *Foodmicrobiology,*25(2008)207-211.
15. E. Vagi, B. Simandi, A. Suhajda, E. Hethelyi, Essential oil composition and antimicrobial activity of *Origanum majorana* L. extracts obtained with ethyl alcohol and supercritical carbon dioxide, *Food research international,* 38 (2005) 51-57.
16. A.P. Raina, K.S. Négi, Essential oil composition of *Origanum majorana* and *Origanum vulgare* ssp. hirtum growing in India, Chemistry of Natural Compounds*,* 47 (2012) 1015-1017.
17. A.T.H. Mossa, G.A.M. Nawwar, Free radical scavenging and anti-acetyl cholinesterase activities of *Origanum majorana* L. essential oil, *Human & experimental toxicology*, 30 (2011) 1501-1513.
18. G. Ben Salha, R.H. Diaz, J. Labidi, M. Abderrabba, Deterpenation of *Origanum majorana* L essential oil by reduced pressure steam distillation, *Industrial Crops & Products*, 109 (2017)116–122.
19. D. Dharajiya, P. Patel, N. Morita, Antibacterial activity of *Emblica officinalis* (Gaertn.) Fruits and *Vitenegundo* (L.) Leaves, *Current Trends in Biotechnology and Pharmacy*, 9 (2015) 357-368.
20. I. Khemiri, B.S. Hedi, N.S. Zouaoui, N.B. Gdara, L. Bitri, The Antimicrobial and Wound Healing Potential of *Opuntia ficus indica* L. inermis Extracted Oil from Tunisia, *Evidence-Bas Complementary and Alternative Medicine*, 2019 Article ID 9148782, https://doi.org/10.1155/2019/9148782
21. S. Matei, A. Matei, G.M. Matei, C.P. Cornea, Utilization of lactic acid bacteria and extracellular compounds in biological control of fungal species, *Research Journal of Agricultural Science*, 47 (2015) 122-132.
22. H. Hajlaoui, H. Mighri, M. Aouni, N. Gharsallah, A. Kadri, Chemical composition and in vitroevaluation of antioxidant, antimicrobial, cytotoxicity and anti-acetylcholinesterase properties of Tunisian *Origanum majorana* L. essential oil, *Microbial Pathogenesis*, 95 (2016) 86-94.
23. R.R. Vera, J.C. Ming, Chemical composition of the essential oil of marjoram (*Origanum majorana* L.) from Reunion Island, *Food Chemistry*, 66 (1999) 143-145.
24. E. Banchio, P.C. Bogino, J. Zygadlo, W. Giordano, Plant growth promoting rhizo bacteria improve growth and essential oil yield in *Origanum majorana* L, *Biochemical systematics and Ecology*36(2008) 766-771.
25. E. Vagi, B. Simandi, A. Suhajda, E. Hethelyi, Essential oil composition and antimicrobial activity *Origanum majorana* L. extracts obtained with ethyl alcohol and supercritical carbon dioxide, *Food Research International,* 38 (2005) 51-57.
26. A. Bejaoui, A. Boulila, M. Boussaid, Chemical composition and biological activities of essential oils and solvent extracts of *Origanum vulgare* subsp. Glandulosum Desf. From Tunisia. *J. Med. Plant. Res*, 7(2013) 2429–2435
27. O. Olfa, A. Mariem, A.M. Salah, B.N.A. Mouhiba, Chemical content, antibacterial and antioxidant properties of essential oil extract from Tunisian *Origanum majorana* L. cultivated under saline condition. Pak, *J. Pharm. Sci*, 29 (2016) 1951-1958.
28. C. Busatta, R.S. Vidal, A.S. Popiolski, A.J. Mossi, C. Dariva, M.R.A. Rodrigues, F.C. Corazza, M.L. Corazza, J.V. Oliveira, R.L. Cansian, Application of *Origanum majorana* L. essential oil as an antimicrobial agent in sausage, Food Microbiol. 25 (2008) 207–211.
29. R.do. S.V. Chaves, R.L. Martins, A.B.L. Rodrigues, E.M. Rabelo, A.L.F. Farias, C.M.C.V. Araujo, T.F. Sobral, A.K.R. Galardo, S.S.M.S.de Almeida, Larvicidal Evaluation of the *Origanum Majorana* L. Essential Oil against the Larvae of the Aedes aegypti Mosquito, *BioRxiv* (2019).

30. H. Hussain, F. Anwar, S. Rasheed, P.S. Nigam, O. Janneh, S.D. Sarker, Composition antioxidant and chemotherapeutic properties of the essential oils from two Origanum species growing in Pakistan, *Rev. Bras. Farmacogn. Braz. J. Pharmacogn*, 21(2011), 943-952.
31. G. Amor, L. Caputo, A. La Storia, V. De Feo, G. Mauriello, T. Fechtali, T, Chemical composition and antimicrobial activity of *Artemisia herba-alba* and *Origanum majorana* essential oils from Morocco, *Molecules,* 24 (2019) 4021.
32. S. Harpaz, L. Glatman, V. Drabkin, A. Gelman, Effects of herbal essential oils used to extend the shelf lifeof freshwater reared Asian sea bass fish (Lates calcarifer), *J Food Prot,* 66 (2003) 410-417.
33. H. Hajlaoui, N. Trabelsi, E. Noumi, M. Snoussi, H. Fellah, R. Ksouri, A. Bakhrouf, Biological activities of the essential oils and methanol extract of two cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine, *World J. Microbiol. Biotechnol*, 25 (2009) 2227-2238.
34. S. Dhaouadi, W. Rouissi, A.M. Hamdane, I. Hannachi, B. Nasraoui, Antifungal activity of essential oils of *Origanum majorana* and *Lavender angustifolia* against *Fusarium* wilt and root rot disease of melon plants, *Tunisian Journal of Plant Protection*, 13 (2018) 39-55.
35. B. Baydar, O. Sagdic, G. Ozkan, T. Karadogan, Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey, *Food Control*,15(2004)169-172.
36. V. Kumar, C.S. Mathela, A.K. Tewari, K.S. Bisht, K.S, In vitro inhibition activity of essential oils from some Lamiaceae species against phytopathogenic fungi, *Pesticide Biochemistry and Physiology*, 114 (2014) 67-71.
37. V.V. Ebani, S. Nardoni, F. Bertelloni, S. Giovanelli, G. Rocchigiani, L. Pistellib, F. Manciantia, Antibacterial and antifungal activity of essential oils against some pathogenic bacteria and yeasts shed from poultry, *Flavour Fragr. J,* 31 (2016) 302–309.
38. S. Inouye, K. Uchida, H. Yamaguchi, T. Miyara, S. Gomi, M. Amano, Volatile Aroma Constituents of Three Labiatae Herbs Growing Wild in the Karakoram-Himalaya District and Their Antifungal Activity by Vapor Contact, *J. Essent. Oil Res*, 13 (2001) 68–72.
39. M.C. Manganyi, T. Regnier, E.I. Olivier, Antimicrobial activities of selected essential oils against *Fusarium oxysporum* isolates and their biofilms. *South African Journal of Botany,* 99 (2015) 115-121.
40. F. Ben Abdallah, R. Lagha, A. Gaber, Biofilm Inhibition and Eradication Properties of Medicinal Plant Essential Oils against Methicillin-Resistant Staphylococcus aureus Clinical Isolates, *Pharmaceuticals*, 13 (2020) 369.