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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OILS AND SOLVENT EXTRACTS OF *ORIGANUM ELONGATUM* FROM MOROCCO

Oumam, Nazha[1] ; Rais, Ayoub[1] ; Benaamara, Ahmed[1] ; Mohamed Abdoul-Latif, Fatouma[2] ; Ayoub, Ainane[3] ; André, Philippe[4] ; Tarik, Ainane[3] ; Abourriche, Abdelmjid[1]

[1] Laboratory of Biomolecular and Organic Synthesis, Faculty of Sciences Ben M'sik, Avenue Cdt Driss El Harti, University Hassan II of Casablanca, BP 7955 Sidi Othman Casablanca, Morocco.

[2] Medicinal Research Institute, Center for Research and Study of Djibouti, BP 486, Djibouti.

[3] Superior School of Technology of Khenifra (EST-Khenifra), University of Sultan Moulay Slimane, BP 170, Khenifra 54000 Morocco.

[4] Laboratory of Biophotonics and Pharmacology UMR CNRS 7213, University of Strasbourg, Faculty of Pharmacy, 74 route du Rhin, CS 60024, 67401 ILLKIRCH Cedex, France.

*t.ainane@usms.ma

Abstract

The present study was performed to investigate the antimicrobial and antioxidant activities of essential oil and solvent extracts obtained from *Origanum elongatum* plant. The chemical composition of *Origanum elongatum* essential oil (spontaneous plant endemic to Morocco's Rif mount) was determined by Gas Chromatography coupled with Mass Spectrometry (GC-MS). The results showed that this plant is characterized by predominance of sabinene (43.92%), followed by γ-terpinene (16.93%), carvacrol (9.71%), isoterpinolene (8.74%) and thymol (5.53%).The antibacterial activity was evaluated against six Gram$^+$ and Gram$^-$ bacteria known for their pathogenicity. The antifungal activity was tested against the yeast *Candida albicans* and three species of phytopathological molds. The results showed a large antimicrobial effect of *Origanum elongatum* essential oil against microbial agents tested. The antioxidant activity was measured employing the method of scavenging of free radical DPPH by essential oil and methanolic extract of *Origanum elongatum*. The results showed that methanolic extract behaved as a strong free radical scavenger providing IC50 value at 83.36 µg/ml which is comparable to the control positive BHT, whereas the essential oil showed weaker activity with IC50 value at 839.11 µg/ml. Total phenolic constituents, based on Gallic Acid Equivalents (GAE), revealed the presence of total soluble phenolics in the extract as 108.33 mg EAG/g DW, and most probably might be responsible for the antioxidant activity of methanolic extract. The results found in this study may suggest that essential oil and extracts by solvents of *Origanum elongatum* plant, possess compounds with interesting antimicrobial properties as well as antioxidant activity, and therefore can be used as natural product in pharmaceutical, cosmetic and food industry.

Keywords: *Origanum elongatum, Essential oil, Antimicrobial activity, Antioxidant activity.*

Introduction

Oregano is an important medicinal plant which belong to the family *Labiatae*. The genus *Origanum* includes 42 species and 18 hybrids widely distributed in the Mediterranean region and Eurasia [1]. Different species of this genus are widespread and growing in abundance on the rocky slopes and mountains areas. The species of the genus *Origanum* have several biological properties. They are widely used in pharmaceutical, cosmetic and food industry thanks to their food flavoring and fragrance properties [2]. Some species are used as fragrance components in cosmetics, pharmaceuticals, soaps, perfumes, detergents and as flavoring agents [3]. Oregano plant is also used as traditional remedy to treat various ailments thanks to its antibacterial, antifungal, insecticidal and antioxidant activities [4-5]. It is used in case of gastrointestinal complaints [6] and as as expectorant, antiparasitic, carminative, stimulant and tonic [7]. Also it is used against colic, cough, toothaches and irregular menstrual cycles [8]. Previous studies reported that oregano plant showed a preventing effect against diabetes complications and has an anti-inflammatory activity [9].

The reported biological properties observed for *Origanum* plants are due to their contents in secondary metabolites such as phenolic components. Essential oils constitute the most active compounds among these secondary metabolites, compared to other extracts obtained by solvents (hexanic, ethanolic, methanolic and aqueous extracts) [10-11]. Recent studies have shown that essential oil as well as their active principles possess several pharmacological properties like antibacterial, antifungal, antioxidant antimutagenic, angiogenic, antiparasitic and other activities [12]. The biological properties of *Origanums* are not due only to their essential oils but also due to their polar constituents which act synergistically with these oils.

The essential oils composition of *Origanum* species is mainly characterized by the predominance of phenolic compounds. Generally, it is carvacrol and/or thymol which predominate, followed by γ-terpinene, p-cymene, linalool, terpinene-4-ol and sabinene hydrate[14]. The second chemotype is characterized by presence basically of monoterpene alcohols, such as terpinen-4-ol, either alone or together with cis- and trans-sabinene hydrate [13-15]. Previous studies has reported relation between the high activity of essential oil of Oregano plants and the presence of phenolic compounds (carvacrol and thymol) and their precursors γ-terpinene and p-cymene [16].

The plant *Origanum elongatum* is one of the species of the *Origanum* genus. It is an indigenous plant of Morocco that grows naturally in the north and center of the country. It is used as a traditional medicinal herb in Morocco to treat various ailments like digestive disorders and menstrual problems, sore throat and also as an expectorant and antitussive. The plant has other applications as culinary herb. It is commonly used as tisane in tea and in flavoring food like tomatoe sauces, vegetables, fish and meat. However, to the best of our knowledge, there have been few studies about the chemical composition of the essential oil of *Origanum elongatum,* and there are no available reports on biological effects of different extracts from this Moroccan endemic plant.

The aim of the present study was to evaluate the endemic Moroccan species *Origanum elongatum,* collected from Ketama region located in the Rif mountains (north of Morocco), and investigate some of the biological activities of *Origanum elongatum*'s essential oil and other extracts obtained by different solvents. This work focused on (i) analyzing the composition of the essential oil by Gas Chromatography coupled with Mass Spectrometry (GC / MS) to determine the chemotype of the essential oil, and (ii) the study of antibacterial and antifungal activities of essential oil and solvent extracts against Gram+ and Gram- bacteria and fungi. (iii) The antioxidant activity of essential oil and methanolic extract of *Origanum elongatum* was investigated. In addition, the amount of total phenol components in the plant was determined.

Materials and methods

Plant material

Origanum elongatum plant is originally from Ketama region located in Rif mountains (north of Morocco). This plant grows naturally at an altitude

of 1200 m. Harvesting was done in flowering stage, during months of June-July. Drying of the plant was carried out for several days without direct exposure to sunlight and with flipping.

Preparation of the extracts

The leaves of dried plant were subjected for 3h to water-distillation using a Clevenger-type apparatus. The obtained essential oil (EO) was dried over anhydrous sodium carbonate and after filtration, stored at 4°C until analysis.

The dried leaves of the plant material were ground. Then the powder (100g) was extracted (500ml) successively using three different solvents with increasing polarity: Petroleum ether, chloroform and ethanol at room temperature for 24 hours each. The extracts obtained were: Petroleum Ether Extract (PE), Chloroformic Extract (CH) and Ethanolic Extract (ET). The three extracts were filtered through Whatman filter paper (No1) then concentrated in a vacuum at 40°C using a rotary evaporator.

GC-MS analysis conditions

The GC-MS analyses were performed on a gas chromatograph (type Varian 450-GC) coupled to a mass spectrometer (type Varian 240-MS) with electron impact ionization (70 eV). DB-WAX capillary column (60m x 0,25mm i.d., 0.25µm film thickness) was used. The incubation temperature of the column was programmed at 60°C for 10 min with an increase of 3°C per minute to 250°C where it was maintained for 10 min. The carrier gas was helium, the volume injected is 1ml and the flow is 1 ml/min. The temperature of the injector and detector were maintained respectively at 220 and 250°C. Mass spectra were taken at 70eV. Mass range was 45-450 m/z. The compounds were identified by comparing their mass spectra with those of standard substances given in the NIST library and those of standard substances given in the literature.

Antimicrobial activity

The antibacterial activity was evaluated against six $Gram^+$ and $Gram^-$ bacteria (*Staphylococcus aureus, Staphylococcus aureus* meticillin-resistant, *Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa*) involved in nosocomial infections and (*Salmonella Aboni*) known for a high frequency to contaminate food. The antifungal activity was investigated against the yeast *Candida albicans* known for its pathogenicity and against 3 species of phytopathogenic molds (*Verticillium dalhaie*, *Phytophthora infestans* and *Fusarium oxysporum f. sp. radicis-lycopersici* (FORL)). The tested bacteria and yeasts belong to the microorganism's collection of Faculty of Pharmacy of Strasbourg-France (Tables 2 and 4). The tested molds belong to to the microorganism's collection of the National Institute of Agricultural Research (INRA) of Meknes-Morocco (Tables 3). The culture media used for bacteria was Agar and Mueller Hinton Broth (MHA and MHB). The Sabouraud Agar (SAB) was used for both molds and yeasts.

Two methods were used to determine the antimicrobial activity of *Origanum elongatum essential oil* : Disc diffusion method (performed against bacteria and fungi) and the microdilution method to determine the Minimum Inhibitory Concentration (MIC) (performed only on bacteria). All tests were performed in triplicate.

The disc diffusion method was applied for the determination of antimicrobial activity of *Origanum elongatum* essential oil. Seeding plates were applied by flooding 2 ml of microbial suspension (10^6 CFU/ml of bacteria, 10^5 CFU/ml of yeast and 10^4 spores/ml of molds) on the surface of agar media (MHA and SAB), then the supernatant was aspirated. After 20 minutes of drying Petri plate at room temperature, a cellulose disc (6 mm of diameter) was impregnated by 10µl of EO for bacteria and fungi. The impregnated disc was applied into the center of the Petri plate.

The extracts PE, CH and ET were respectively dissolved using the solvents Petroleum Ether (PE), Chloroform (CH) and Ethanol (ET). The quantity deposited per disc was 500 µg/disc. The plates were then deposited in the refrigerant for 2 hours at 4°C to allow better diffusion of essential oils and extracts. Standard discs of the antibiotic Nitrofurantoin (FT: 300 µg/disc) and Cefotaxime (CTX: 30 µg/disc) served respectively as the positive antibacterial control. The solvents (petroleum ether, chloroform and ethanol) were used as a negative control and tested in the same conditions as the extracts. The plates were incubated at 37°C for 24h for bacteria, at 28°C for 24h for yeasts and at 26°C

for 4 days for molds. The diameters of the inhibition zones were measured after inoculation. Antimicrobial activity was assessed by measuring the inhibition zone.

From a pure culture of tested bacteria we prepared a bacterial suspension of concentration estimated at 10^6 CFU/ml. The culture medium used for the preparation of the bacterial suspension was Mueller Hinton Broth (MHB). Essential oil and solvent extracts (PE, CH and ET) were successively diluted to one-half in DMSO, by preparing a serial dilution ranging from 0.25% (1600 µg/ml) to 0.016% (100 µg/ml) for essential oil and other successive serial dilution ranging from 1200 µg/ml to 75 µg/ml for PE, CH and ET extracts.

The concentration of DMSO in the wells did not exceed 5%. The MIC values of essential oil against the bacterial strains tested were determined by the microdilution method using 96-well sterile plates. These plates were prepared by depositing, in all wells 190 µl of bacterial suspension at 10^6 CFU/ml and then by adding 10 µl of five dilutions prepared of the essential oil or solvent extracts in the first five wells and 10 µl of sterile distilled water in the last well without containing the essential oil. This latter well serves as a negative control. Two wells were used for each concentration. The final volume of each well is 200 µl. The plates were incubated at 37 °C for 24h.

The optical density (OD) was measured at 620 nm after 24 h of incubation of plates at 37°C, in order to determine bacterial growth in each well. The test was performed in triplicate for each concentration of the essential oil. The plates were shaken well before each reading to ensure a good homogenization. The MIC is defined as the lowest concentration of essential oil which caused inhibition of bacterial growth manifested by an absence of bacterial trouble.

Determination of total phenols

The extract of total phenolic compounds was extracted from *Origanum elongatum* powder as described by Mhada et al. (2020) [17] slightly modified. The powder sample (1 g) was extracted twice with 25 mL of methanol. The two volumes were combined and made up to 50 mL. The methanolic extract obtained was filtered and stored at 4 °C in the dark for analysis.

Total phenol contents in the extracts were determined following the method adapted from Espada-Bellido et al. (2017) [18] slightly modified, using Follin-Ciocalteu reagent. Gallic acid was used as a standard for the calibration curve. The sample (1 mL) and 4 mL of sodium carbonate (75 g/L) were added to 5 mL of Folin-Ciocalteu reagent (diluted in distillated water 1/10). After 30 min of reaction at 40 °C the absorbance was measured at 765 nm (Spectrophotometer Uviline 9400 UV-Visible). The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW). Tests were carried out in triplicate.

Antioxydant capacity

50g of powder leaves of *Origanum elongatum* were extracted with methanol solvent at room temperature (2x200 mL) for 24 hours. The methanolic extract was filtered through Whatman filter paper (No1), subsequently it was evaporated to dryness under reduced pressure at 40°C.

The radical scavenging activity of essential oil and methanolic extract of *Origanum elongatum* was evaluated according to the slightly modified method of Radan et al. (2018) [19]. A volume of 10 µL of various concentrations of essential oil, methanolic extract and Butylhydroxytoluene (BHT) were added separately to 300 µL of a 0.037 mg/mL methanol solution of DPPH (Diphenylpicrylhydrazyl). After a 30 min incubation period in the dark at room temperature, absorbance was read against a blank at 517 nm and was compared with a control without extracts. A blank was prepared for each sample using methanol instead of the DPPH solution. BHT was used as reference compound.

The decrease in optical density of DPPH on addition of the test samples in relation to the control was used to calculate the antioxidant activity as percentage of inhibition (% IP) of DPPH radical :

$$\% IP = (A_{control} - A_{sample} / A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of DPPH radical + sample (EO or standard). The antioxidant

activity of samples was expressed as IC_{50} in µg/mL required to inhibit the formation of DPPH radicals by 50%. IC50 values were determined from the plotted graphs of scavenging activity against the concentration of the extracts. A low IC_{50} value represents a high antioxidant activity. Triplicate measurements were carried out.

Results and Discussion

Chemical composition of essential oil

The analysis by Chromatography Gas coupled with Spectrometry of Mass (GC-MS) of *Origanum elongatum* essential oil (Table 1) showed the presence of several compounds, and among them 18 that are predominant and represent (99.84 %) of this oil. Other compounds are present in trace amounts. The first five major compounds are: sabinene (43.92 %), followed by γ-terpinene (16.93 %), carvacrol (9.71 %), isoterpinolene (8.74 %) and thymol (5.53 %). Regarding the composition of the essential oil of Moroccan *Origanum elongatum*, there are few studies that were interested to this plant among which the studies of [20-23] These studies have reported that the essential oil of *Origanum elongatum* has a carvacrol chemotype. This phenolic component is the predominant compound followed by p-cymene, γ-terpinene in smaller quantities. Benjilali (1986) determined the carvacrol with a percentages of (36.6 to 76.6 %), Velasco-Negueruela (1991) with a percentages of (21.2 to 17.6 %). In Figueredo and al., (2006) study, the carvacrol was the predominant compound with a percentage of (79.2 %) followed by γ-terpinene (3.7 %), p-cymene (5.2 %) and linalool (2.4 %). So in comparison with previous studies, the present study has identified a different composition of essential oil of Moroccan *O. elongatum,* in which the phenolic compounds (carvacrol and thymol) are in minority and with the predominance of sabinene.

Antimicrobial activity

The antimicrobial activity of *Origanum elongatum* against bacteria tested in this study was evaluated by comparing the inhibition diameters and MIC values for essential oil (EO) and solvent extracts. The results of disc diffusion method and microdilution method, given in the Tables 2 and 4, showed that essential oil of *Origanum elongatum* presents a wide spectrum antibacterial activity against tested bacteria with inhibition diameters and MIC values that vary from species to other. The inhibition zones and MIC values of EO were in the range of 16.5 – 43 mm and 200 – 400 µg/mL, except for *Pseudomonas aeruginosa* bacterium that seems most resistant to the bactericidal effect of oregano essential oil (11 mm in diameter of inhibition and 3200 µg/mL as MIC) compared to the other bacteria. The same result was observed in the results of previous studies in which they mentioned that *Pseudomonas* and especially *Pseudomonas aeruginosa* appears less sensitive to the effect of essential oils.

We also found that $Gram^+$ bacteria (*S. aureus* and *S. aureus* methicillin-resistant) have inhibition diameters that are more important compared to other $Gram^-$ bacteria. This result is in agreement with previous studies, obtained on agar medium, that studied the action of essential oils against pathogenic bacteria responsible for foodborne diseases. The results showed that essential oils were more active against $Gram^+$ bacteria compared to $Gram^-$ that seem more resistant [24]. This resistance could be explained by the wall structure of $Gram^-$ bacteria which is richer in proteins and lipopolysaccharides than those of $Gram^+$ which makes it more hydrophilic, so it prevents the hydrophobic terpenes to adhere to the bacterial wall [25]. However we could find exceptions as in the case of *E. coli* which present the highest sensitivity among the $Gram^-$ bacteria against *Origanum elongatum* essential oil. This result was also observed in the study of Hays et al. (1997) [26] in which they found that the bacterium *E. coli* is more sensitive against oil extracted from tea tree compared to the $Gram^+$ bacterium *Staphylococcus aureus*.

Regarding the antibacterial activity of solvent extracts of *Origanum elongatum*, the results of disc diffusion method (Table 2) show that Petroleum ether (PE) and Chloroformic (CH) extracts are more active than Ethanolic (ET) extract which present a weak antibacterial effect against the bacteria tested. The extracts (PE and CH) are active only against Gram+ bacteria compared to Gram- bacteria. These results confirmed that bacteria Gram+ are

more sensitive than bacteria Gram- to the antibacterial activity of plant extracts.

The microdilution method was tested only for PE and CH extracts (Table 4) which present a higher antibacterial effect than ET extract, and only against bacteria Gram+. The results obtained from the microdilution method confirmed the sensitivity of Gram+ bacteria to the extracts PE and CH.

With regards to the antifungal activity (Table 3), the inhibition zones for the yeast and fungi species tested, that are sensitive to the essential oil of *Origanum elongatum* were in the range of (34 - 43.5 mm). The results obtained by disc diffusion method, showed that the essential oil present an important inhibitory effect against all fungi species tested, with a higher sensitivity of *Candida albicans* species (43.5 mm). Based on the same results (Table 3), it is possible to conclude that the EO has stronger and broader spectrum of antifungal activity as compared to PE, CH and ET extracts.

If we compare both the results of antimicrobial activities of EO and solvent extracts (PE, CH and ET) of *Origanum elongatum*, it is possible to conclude that the antimicrobial effect of EO of this plant present a stronger and broader spectrum of antibacterial and antifungal activities as compared to those showed by PE, CH and ET extracts of *Origanum elongatum.* This might be explained by the presence of compounds in EO which are more antimicrobial as compared to the other extracts. This observation was confirmed in the previous studies which have mentioned that essential oils contain more antimicrobial substances than other extracts obtained by solvents such as water, methanol, ethanol and hexane [27-28].

In general, essential oils of *Origanum* species contain mainly aromatic monoterpenes such as carvacrol and thymol, and their antimicrobial activity is often attributed to these compounds [29-30]. In our study we identified a chemical profile of *Origanum elongatum* essential oil with sabinene (43.92%), where the phenolic compounds are in minority. So the antimicrobial activity observed against the different pathogens studied could be attributed to the synergistic effect of major compounds of the essential oil with minor constituents.

Total phenolic compounds

Phenolic compounds such as flavonoids, phenolic acids, and tannins are widely distributed in plants, and in recent years they have gained much attention due to their antioxidant activity and free radical-scavenging ability with potential beneficial implications in human health [31]. Based on the absorbance value of the methanolic extract solution, reacted with Folin-Ciocalteu reagent and compared with standard solutions of Gallic acid equivalents as described previously, the amount of total phenolic compounds was estimated as 108.33 mg EAG/g of dry extract.

Antioxidant activity

The DPPH (Diphenylpicrylhydrazyl) is a stable radical, which has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts and foods [32-33]. The effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable molecule. The absorption maximum of a stable DPPH radical in methanol was 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, which is attributed to the ongoing reaction between antioxidant molecules and the free-radical, results in the scavenging of the radicals by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidant activity of antioxidants [34]. The DPPH free radical scavenging activity of essential oil and methanolic extract of *Origanum elongatum* has been shown in Table 5. The concentration of the sample necessary to decrease the initial concentration of DPPH by 50% (IC50) under the experimental condition was calculated. IC50 values of essential oil and methanolic extract were compared with the standard BHT.

As shown in Table 5, the DPPH radical scavenging activities of methanolic extract and the reference control (BHT) were remarkable, exhibiting ability to reduce the stable radical DPPH to yellow-colored diphenylpicrylhydrazine with IC50 values of 83.36 µg/mL for methanolic extract and 73.86 µg/mL for

BHT, whereas essential oil exhibits a low DPPH radical scavenging activity (IC50 value of 839.11 µg/mL). This superiority of the methanolic extract could be attributed to the presence of phenolic compounds in the plant which represent 108.33 mg EAG/g DW. This important activity could be due also to synergistic effects of phenolic acids (rosmarinic acid) and polyphenols as well as other chemicals like flavonoids [35]. The low antioxidant activity of essential oil could be related to its chemical composition that showed a low level of phenols. Previous studies have confirmed that phenolic compounds (especially thymol and carvacrol) of essential oil from several *Origanum* species, exhibit the highest antioxidant activity compared to the other compounds [36]. Our results concerning the radical scavenging activity are in agreement with those of previous reports, since the percentage of thymol and carvacrol were low in *Origanum elongatum* essential oil. Therefore, essential oil exhibits a low DPPH radical scavenging activity compared to methanolic extract.

Conclusion

The results of this study show that essential oil of *Origanum elongatum* (endemic plant to Morocco's Rif mount) present chemical profile with the predominance of sabinene (43.92%) while phenolic compounds (carvacrol and thymol) are in minority. The different extracts obtained from *Origanum elongatum* plant exhibited interesting antibacterial, antifungal and antioxidant activities. These results may suggest that *Origanum elongatum* posses compounds with different biological properties that can be used as natural product in pharmaceutical or natural therapies against infectious diseases in human beings and plants. The findings in this study are supporting the use of *Origanum elongatum* plant as additive in food and as part of traditional remedies for the treatment of infectious diseases.

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Table 1. *Origanum elongatum* essential oil composition.

Peak	TR (min)	Components	Composition (%)
1	12.476	α-pinene	4.32
2	13.594	Camphene	0.51
3	14.933	3-carene	0.36
4	16.259	α-pinene	0.25
5	16.743	β-Phellandrene	3.23
6	17.311	Isoterpinolene	8.74
7	17.941	Limonene	1.15
8	18.294	β-terpinene	0.74
9	19.778	γ-terpinene	16.93
10	20.807	Sabinene	43.92
11	21.018	Terpinolene	1.16
12	26.118	p-cimene	0.28
13	26.443	1-propylcyclopentanol	0.33
14	27.126	Cis-2-p-menthen-1-ol	0.37
15	29.628	α-terpineol	1.23
16	31.626	Caryophyllene	1.08
17	46.084	Thymol	5.53
18	46.771	Carvacrol	9.71

Table 2. Antibacterial activity of *Origanum elongatum* essential oil and solvent extracts against $Gram^+$ and $Gram^-$ bacteria based on disc diffusion method.

Bacterial species	Essential oil (10 µl/disc)	PE extract	CH extract	ET extract	Standard antibiotic discs[a]
	Inhibition zone diameter (mm)				
Staphylococcus aureus (ATCC 6538209P)	43	16	14	7.5	26 (FT)
Staphylococcus aureus meticillin-resistant (T32370)	32.5	12	10	8	26 (FT)
Klebsiella pneumoniae (A9d)	16.5	6	6	6	32 (CTX)
Salmonella Aboni (CIP 8039)	23	6	6	6	36 (CTX)
E. coli (CIP 53126)	26.5	6	6	6	35.5 (CTX)
Pseudomonas aeruginosa (CIP 76110)	11	6	6	6	32 (CTX)

[a] FT : Nitrofurantoin (300µg), CTX : Cefotaxime (30µg)
The diameter of the disc used = 6 mm

Table 3. Antifungal activity of *Origanum elongatum* essential oil and solvent extracts against the fungi based on disc diffusion method.

Yeast and fungi species	Essential oil (10 µl/disc)	PE extract	CH extract	ET extract
	Inhibition zone diameter in (mm)			
Candida albicans	43.5	9.5	6	6
Phytophthora infestans	34.5	6	6	6
Verticillium dahliae	34	6	6	6
Fusarium oxysporum f.sp.radicis-lycopersici (FORL)	40	6	6	6

Table 4. MIC values of *Origanum elongatum* essential oil and solvent extracts (PE and CH) against bacteria tested in microdilution assay.

Bacterial species	Essential oil CMI (µg/mL)	PE extract CMI (µg/mL)	CH extract CMI (µg/mL)
Staphylococcus aureus (ATCC 6538209P)	200	1200	1200
Staphylococcus aureus meticillin-resistant (T32370)	200	1200	1200
Klebsiella pneumoniae (A9d)	400	-	-
Salmonella Aboni (CIP 8039)	400	-	-
E. coli (CIP 53126)	200	-	-
Pseudomonas aeruginosa (CIP 76110)	3200	-	-

Table 5. Effects of *Origanum elongatum* essential oil and methanolic extract and positive control BHT on the free radical scavenging (DPPH).

Sample	DPPH, IC50 (µg/mL)
Essential oil	839.11
Methanolic extract	83.36
BHT	73.68