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Research Article

Evaluation of Chemical Composition and Biological Activities of Essential Oil and Methanolic Extract of *Origanum vulgare* L. *ssp. glandulosum* (Desf.) *Ietswaart* from Algeria

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ABSTRACT

The objective of this study was to evaluate the chemical composition of *O. vulgare L. ssp. glandulosum (Desf.) Ietswaart* essential oil (EO), to determine total phenolic constituent in methanolic extract and investigate the antibacterial and antioxidant activities of essential oil and methanolic extract. For antibacterial activity, susceptibility tests were expressed as inhibition zone by the disc diffusion method and minimal inhibitory concentration (MIC) by broth microdillution method. Antioxidant activity was evaluated using DPPH scavaging capacity assay. GC and GC/MS analyses of the oil resulted in the identification of 43 compounds, representing 98.55% of the oil; para-cymene (25.615%), thymol (23.129%), carvacrol (20.321%) were the main components. The methanolic extract showed total phenolic contents of 526.75 µgGAE/mg. EO was particularly found to possess strong antibacterial capacity while methanolic extract remained inactive. In antioxidant activity, methanolic extract exhibited very high scavenging ability on DPPH radicals with IC50= 25.59 µg/ml whereas EO presented an IC50= 461.62µg/ml.

INTRODUCTION

The essential oils and various extracts of plants have attracted considerable academic attention as well as industrial interest due to their various properties, particularly, the antimicrobial and antioxidant activities. The plant oils and extracts have formed the basis of many applications because of the resistance that pathogenic microorganisms have built against antibiotics[1] and the increasing popular concern about the safety of food and the potential toxicities of the synthetic antioxidants on health[2]. There are several food plants (spices in particular) with a significant volatile organic compounds content (e.g *Levisticum officinale, Origanum vulgare, O. majorana, Cinnamomum ssp., Zingiber ssp., Citrus ssp., Elettaria cardamomum, Foeniculum vulgare, Salvia officinalis, Syzygium aromaticum, Pimpinella anisum* and *Ocinum ssp*., to name a few examples)[3].

Essential oil composition may vary considerably between plant species and varieties, and, within the same variety, from different geographical origin influenced by soil and environmental conditions[3]. Essential oils are usually characterized by two or three major components at quite high concentrations (up to 80%) compared to other components present only in trace amounts[5].

The essential oils and some of their components can often be used in different forms for human consumption. It was observed that potato starch or sunflower oil concentrations of above 5%, reduced the efficacy of oregano and thyme essential oils on Listeria monocytogenes[6]. Their use in foods as preservatives is limited because of flavor considerations. Hence, for the study of biodiversified application, a complete investigation including the chemical composition, efficacy as antibacterial and/or antioxidant agents of an essential oil and/or extract was considered important.

Origanum vulgare L. (oregano), without focusing on specific subspecies, is the most widespread and known species of Lamiaceae family. It is an important aromatic plant widely used in many countries for seasoning foods[7]. *Origanum vulgare ssp. glandulosum (Desf.) Ietswaart*, synonymous *O. glandulosum Desf.*, is an endemic spontaneous plant, growing in North Africa (Algeria and Tunisia)[8]. In Algeria, *O. glandulosum Desf.* is an aromatic shrub called ‘‘zaâter” which is mostly used as a medicinal plant against whooping cough, cough, fever and bronchitis[9].

Carvacrol and thymol, the two main phenols that constitute about 78–82% of the essential oil of oregano, are principally responsible of its antibacterial and antioxidant activities[10, 11]. In addition, other minor constituents such as γ-terpinene and ρ-cymene, two monoterpenes hydrocarbons that constitute about 5 and 7% of the total oil, respectively, also contribute to these activities[10, 12].

In recent years, the essential oil of *Origanum* species have intensively been investigated for their chemical composition and potential use as natural antimicrobial and antioxidant agents all over the world: from France[13], Austria[14], Southern Italy[15], China and Pakistan[16], Bosnia[17], Bulgaria[18], Lithuania[19], Tunisia[20] and different Mediterranean populations[21].

As far as our literature survey could ascertain chemical composition and antibacterial activities of *O. vulgare* from

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east of Algeria have been previously published. Amrouni et al.[22] have studied antibacterial activity on nosocomial bacteria : *Pseudomonas aeruginosa*, and reference strains: *Staphylococcus aureus* and *Escherichia coli*, but no information is available on the action on other microorganisms, antioxidative nature of this plant, and its potential use as natural foods preservative.

The chemical composition of Algerian *O. glandulosum Desf.* essential oil have been studied[23, 24, 25, 26, 27, 28] and all reports have identified thymol and carvacrol as the main components. Antimicrobial and antioxidant activity of the essential oils have been proved[23, 25, 28]. However, there is no report on the biological activities of methanolic extract of *O. glandulosum Desf.*

Therefore, due to high utilization of the endemic *O. glandulosum Desf.* by local population to treat many diseases we were interested in this plant. For this reason, the aims of the present study was: to analyze the chemical composition of a hydrodistilled essential oil of *O. glandulosum Desf* collected from Guelma city in east Algeria by a GC/FID and GC/MS system in order to determine the essential oil chemotype and compare it with oregano essential oil from other localities of Algeria and world region; to determine total phenolic constituent in methanol extract and investigate the antimicrobial and antioxidant activities of both essential oil and methanol extracts from *O. glandulosum Desf.*

MATERIALS AND METHODS

Plant material

O. glandulosum Desf. plants at flowering stage were collected from Nechmaya region of Guelma city (north-eastern part of Algeria) in June 2012. The aerial parts (leaves and flowers) were dried in the shade at room temperature.

Preparation of the extracts

Isolation of the essential oil

The air-dried and ground flowering parts of the plants were submitted for 2 hours to water-distillation, using a Clevenger-type apparatus. The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4°C until tested and analyzed. Yield based on dried weight of the sample was calculated.

Preparation of the methanol extracts (MeOH)

The air-dried and finely ground samples were extracted with methanol (99.7%) by using a Soxhlet apparatus for about 6 hours[29]. The methanolic extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuo at 40° C using a rotary evaporator. The residues obtained were stored in a freezer until further tests.

GC and GC-MS analysis

GC/FID

The GC-FID analysis was performed on a Hewlett-Packard 6890 gas chromatograph equipped with a DB5 MS column (30 m X 0.25 mm, 0.25 µm, Agilent Technologies, USA)

and fitted to FID (Flame Ionisation Detector). The injector and detector were operated at 280 and 300°C, respectively. Oven temperature program was 5 min isothermal at 50°C then raised to 300°C at a heating rate of 5°C/minute, and finally isothermally held for 5 minutes. As a carrier gas, Hydrogen at 1.0ml/min was used. 1µL of the essential oil diluted in hexane (1/30) was injected in a split mode in the ratio of 1: 60. The percentage of composition of the essential oil was calculated by electronic integration of FID peak areas.

GC/MS

The analyses of the volatile constituents were run on a Hewlett-Packard GC-MS system: Gas Chromatograph Model 7890 coupled to a 5975 Mass Selective Detector; and equipped with a DB5 MS column (20 m X 0.18 mm, 0.18 µm, Agilent Technologies, USA). The injector and detector were operated at 280 and 300°C, respectively. Oven temperature was programmed (50°C for 3.2 minutes, then 50 to 300°C at 8°C/minute and subsequently, held isothermally for 5 minutes). As a carrier gas, Helium with a flow rate of 1.0ml/minute was used. 1µL of the essential oil diluted in hexane (1/30) was injected in a split mode in the ratio of 1:250. The MS working in electron impact mode at 70 eV; and anionization energy of 1800 V; ion source temperature, 230°C; mass spectra data were acquired in the scan mode in *m/z* range 33-550.

The identification of components was based on comparison of retention time of each component (Rt) and their mass spectra with those of Wiley 275 mass spectra and NIST (National Institute of Standards and Technology) libraries and those described by Adams[30]. Also, a homemade MS library with the spectra corresponding to pure substances and components of known essential oils was used.

Antimicrobial activity

Microbial strains

The essential oil and extracts were individually tested against a panel of microorganisms, including: five Laboratory reference strains obtained from the American Type Culture Collection: Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC 27853; nine clinical isolates: Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Serratia marcescens, Proteus vulgaris, Proteus mirabilis, Salmonella thyphimurium, Acinetobacter baumanii, Pseudomonas aeruginosa and one foodborne pathogen Klebsiella oxytoca.

Antimicrobial screening

Disc diffusion method

The dried plant extracts were dissolved in the same solvent (methanol) to a final concentration of 30 mg/ml. The agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oil and methanolic extract according to Vuddhakul et al.[31]with slight modification. For the experiments, a loopful of the bacterial working stocks were enriched on a tube containing 9 ml of Mueller-Hinton broth, then incubated at 37°C for 18–24 hours. The overnight cultures were used for the antibacterial activity of the essential oil and extract used in this study and the optical density was adjusted at 0.5McFarland turbidity standards. The inocula of the respective bacteria were streaked onto MHI agar plates

using a sterile swab. After inoculum absorption by agar, sterile filter discs (diameter 6 mm, Whatman paper N°1) were impregnated with 5µl of essential oil or the 30 mg/ml extracts (150 µg/disc) and placed on the inoculated agar, using forceps dipped in ethanol and flamed, as described previously by Gulluce et al.[32]. Standard disc of Gentamycin (10 µg/disc) and blank discs (impregnated with methanol) were used as positive and negative controls, respectively. All Petri dishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the essential oils. And kept at 4°C for 2 hours, and then incubated at 37°C for 24 hours. After the incubation period, the mean diameter of inhibition halo where test microorganism did not grow (clearly visible inhibition zone) was measured in millimeters and recorded as the mean standard deviation (SD). All tests were performed in triplicate

Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC)

The minimal inhibition concentration (MIC) values were studied for the bacterial strains which were sensitive to the essential oil and/or extracts in disc diffusion assay. The inocula of the bacterial strains were prepared from 12 hours broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Essential oil, which was dissolved in 10% dimethylsulfoxide (DMSO), was first diluted to the highest concentration (50 mg/ml) to be tested, and then serial two-fold dilutions were made in a concentration range from 0.048 to 50 mg/ml in 5 ml sterile test tubes containing nutrient broth. MIC values of *O. vulgare* essential oil against bacterial strains were determined based on a microwell dilution method[32].

The 96-well plates were prepared by dispensing into each well 95 µl of nutrient broth and 5 µl of the inoculum. A 100 µl aliquot from the stock solutions of *O. vulgare* essential oil initially prepared at the concentration of 50 mg/ml was added into the first wells. Then, 100 µl from the serial dilutions were transferred into ten consecutive wells. The last well containing 195µl of nutrient broth without essential oil and 5 µl of the inoculums on each strip was used as negative control. The final volume in each well was 200 µl. The plates was covered with a sterile laboratory parafilm and then incubated at 37°C for 18–24 hours. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom. As an indicator of microorganism growth, 10 µl of 2 mg/ml nitroblue tetrazolium (NBT) dissolved in water were added to the wells and incubated at 37 °C for 30 min[33]. The nitroblue tetrazolium (NBT) salt acts as an electron acceptor and the yellow colored NBT is reduced to a purple-blue formazan product by biologically active organisms (Viable bacteria). The MIC was defined as the lowest concentration essential oil to inhibit the growth of the microorganisms. The MBC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no development of turbidity and without visible growth. All tests were performed in duplicate.

Antioxidant activity

Determination of total phenolic contents

Total phenolic constituents of the methanolic extracts of *O. glandulosum Desf.* were determined by the literature methods involving the Folin–Ciocalteu reagent and gallic acid as standard[34]. 0.1 ml of extract solution, containing 1000 µg extract, was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent were added, and flask was shaken thoroughly. After 3 minutes, 3 ml of a solution of 2% Na_2CO_3 were added and the mixture was allowed to stand for 2 hours with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated for all standard gallic acid solutions. The concentration of total phenolic compounds in the methanolic extract was determined as µg of gallic acid/mg dry plant material by using the regression equation that was obtained from the calibration curve of the gallic acid standard. All tests were carried out in triplicate, and gallic acid equivalent values were reported as X ± SD of triplicates.

DPPH radicals scavenging capacity assay

The ability of the plant essential oil and extract to scavenge diphenylpicrylhydrazyl (DPPH) free radicals was assessed using the method described by Takao et al.[35], along with antioxidant activity index (AAI). The stock solution of the plant essential oil and extract was prepared in methanol to achieve the concentration of 2000 µg/ml. Further, two-fold dilutions were made to obtain concentrations from 1000 µg/ml to 7.81 µg/ml. Diluted solutions of extract (2 ml each) were mixed with 2 ml of DPPH methanolic solution (80 µg/ml). After 30 minutes in darkness at room temperature, the absorbance was read in a spectrophotometer at 517 nm. The control samples consisted of 2 ml of methanol added to 2 ml of DPPH solution. Ascorbic acid was used as a positive control. The experiment was performed in triplicate. Scavenging activity is expressed as the inhibition percentage calculated using the following equation:

Scavenging activity (%) = $100 \times [(A_{control} - A_{sample}) / A_{control}]$

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the extract.

The IC_{50} value is the effective concentration at which 50% of DPPH radicals were scavenged. It was obtained from the graph of scavenging activity (%) versus concentration of samples. Low IC_{50} value indicates strong ability of the extract to act as DPPH scavenger.

The antioxidant activity was expressed as the antioxidant activity index (AAI), calculated using the following equation:

AAI = final concentration of DPPH (µg/ml) / IC_{50} (µg//ml)

The estimation of AAI was: if AAI < 0.5 → poor antioxidant activity; AAI > 0.5-1 → moderate antioxidant activity; AAI > 1-2 → strong antioxidant activity and AAI > 2 →very strong antioxidant activity.

DPPH assay on TLC

This procedure was applied for extracts and the essential oil of *O. glandulosum Desf.* following Tepe et al.[36]. Five microlitre of a 1:10 dilution of the extracts in methanol were applied to the TLC plate and methanol – ethyl acetate (1:1) mixture was used as developer. The plate was sprayed with a 0.2% DPPH reagent in methanol and left at

Table 1: Chemical composition of *O. glandulosum Desf.* essential oil.

	RT[a]	Compounds[b]	% FID	KI[c]
1	5,78	Alpha-Thujène	0,895	924
2	5,92	Alpha-Pinène	0,716	932
3	6,25	Camphène	0,12	946
4	6,71	Sabinène	0,018	696
5	6,8	Béta-Pinène	0,154	974
6	6,87	Octène-3-ol	0,52	974
7	6,97	Octanone-3	0,161	979
8	7,04	Myrcène	1,494	988
9	7,35	Alpha-Phellandrène	0,188	1002
10	7,38	Delta-3-Carène	0,072	1008
11	7,55	Alpha-Terpinène	1,787	1014
12	7,72	Para-Cyméne	25,615	1020
13	7,77	Limonène	0,403	1024
14	7,8	Béta-Phéllandrène	0,207	1025
15	7,83	Eucalyptol	0,05	1031
16	7,89	(Z)-Béta-Ocimène	0,074	1032
17	8,08	(E)-Béta-Ocimène	0,06	1044
18	8,31	Gamma-Terpinène	16,612	1054
19	8,5	Cis-Hydrate de Sabinène	0,197	1065
20	8,74	Terpinolène	0,065	1086
21	8,83	Para-Cyménène	0,066	1089
22	8,99	Linalol	0,87	1095
23	9,05	Trans-Hydrate de Sabinène	0,12	1098
24	9,78	Camphre	0,012	1141
25	10,17	Bornéol	0,214	1165
26	10,3	Terpinène-4-ol	0,311	1174
27	10,39	Para-Cymène-8-ol	0,216	1179
28	10,53	Alpha-Terpinéol	0,386	1186
29	10,99	Thymol méthyl-Ether	0,051	1232
30	11,12	Carvacrol méthyl-Ether	0,18	1241
31	11,71	Isomère Thymol MW 150	0,163	1258
32	11,87	Thymol	23,129	1289
33	11,99	Carvacrol	20,321	1298
34	13,58	Béta-Caryophyllène	0,848	1417
35	13,75	Thymohydroquinone	0,487	1553
36	14,02	Alpha-Humulène	0,047	1452
37	14,62	Béta-Bisabolène	0,283	1505
38	14,7	Gamma-Cadinène	0,018	1513
39	14,75	Delta-Cadinène	0,025	1522
40	14,81	Béta-Sesquiphellandrène	0,539	1521
41	14,99	(E)-Alpha-Bisabolène	0,422	1529
42	15,46	Spathulénol	0,02	1577
43	15,54	Oxyde de Caryophyllène	0,41	1582
	Total		98,546	

Notes :

[a] Retention time (as minutes).

[b] Compounds listed in order of their elution

[c] Kovats Index

room temperature for 30 minutes. Yellow spots formed from bleaching of purple colour of DPPH reagent, were evaluated as positive antioxidant activity.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

Yield of the essential oil obtained by hydrodistillation from the aerial part of *O. vulgare* was 2.52 (w/w). As shown in Table 1, GC/MS analysis resulted in the identification of forty three compounds representing 98.546% of the oil. The major constituents of the oil were para-cymene (25.615%), thymol (23.129%) and carvacrol (20.321%). Gamma-terpinene (16.612%) and alpha-terpinene (1.787%) were also present at significant concentrations. Amrouni et al.[22] reported a different composition for this essential oil which showed a carvacrol chemotype with 33.85% carvacrol, 23.64% thymol and 20.85% para-cymene.

Table 2: Antimicrobial activities of *Origanum vulgare L.* essential oil and methanolic extract against the bacterial strains tested

Test microorganisms	Essential oil Plant			extract (MeOH)			Antibiotic	
	IZ[a]	MIC	MBC	IZ[b]	IZ[c]	MIC	IZ[d]	MIC
Gram -								
Acinetobacter baumanii	55.33±1.25	0.78	0.78	9	9	-	54	-
Escherichia coli	49.66±1.15	0.78	0.78	9	9	-	16	-
Escherichia coli ATCC 25922	54.83±1.25	0.78	0.78	8	8	-	31	-
Klebsiella oxytoca	59±1.73	0.78	0.78	7	7	-	30	-
Klebsiella pneumoniae	46.66±1.52	1.56	1.56	7	7	-	32	-
Klebsiella pneumoniae ATCC 700603	57.16±3.25	1.56	3.125	8	8	-	28	-
Proteus mirabilis	59±2.64	1.17	1.17	9	9	-	31	-
Proteus vulgaris	43.66±1.44	0.78	0.78	8	8	-	29	-
Pseudomonas aeruginosa	8.33±1.15	>50	>50	8	8	-	35	-
Pseudomonas aeruginosa ATCC 27853	15±1.73	6.25	6.25	7	7	-	33	-
Salmonella thyphimurium	50.25±1.06	1.56	1.56	7	7	-	19	-
Serratia marcescens	47.33±3.21	1.56	1.56	9	9	-	18	-
Gram +								
Enterococcus faecalis ATCC 29212	51.83±2.46	0.78	0.78	8	8	-	31	-
Staphylococcus aureus	63.33±3.05	1.56	1.56	12	6	-	28	
Staphylococcus aureus ATCC 25923	51.83±2.56	0.78	0.78	8	8	-	29	-

Notes :

IZ[a] = Inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 5µl of essential oil.

IZ[b] = Inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 150 µg/disc.

IZ[c] = Inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 5 µl of methanol.

IZ[d] = Diameter of disc diffusion (mm); Gentamycin (10µg/disc) were used as positive reference standards antibiotic discs

MIC = Minimal inhibitory concentrations as (mg /ml)

Except Egyptian *Origanum vulgare* essential oil with an important concentration of Pulegone[37], most essential oils from southern Mediterranean presented four main components with different percentages : carvacrol, thymol, ρ-cymene and γ-terpinene[20, 23, 24,38].

Algerian *O. glandulosum Desf.* From Jijel[27], Constantine[27], Setif [25,26] and Tlemcen[28] presented a low ρ-cymene content compared to our essential oil (7.5%, 6.6%, 14.6%, 7.9% and 17,1% respectively). And all showed a thymol and/or carvacrol chemotype. However it is important to note that the compositions of these essential oils obtained by hydrodistillation may vary from one region to another. This variability concerns particularly carvacrol for which food manufacturers have a particular interest.

To the best of our knowledge, there are many reports on the chemical composition of the essential oil isolated from different *O. vulgare* subspecies from different regions. Most of them indicate the presence of two main chemotypes of this essential oil, one contains as major components the phenols thymol and/or carvacrol[39, 40] and other consists mainly monoterpene alcohols[41, 42].

It is known that *O. vulgare* species presents great variability in its essential oil composition due to the existence of different subspecies, but also to a numerous of parameters where mainly are the local climatic, seasonal, geological and geographical factors[43].

However, the chemical composition of the studied essential oil differ completely with those previously reported on the literature and displayed a different specific oil chemical profile with para-cymene, thymol and carvacrol as dominant components.

Antimicrobial activity

The antimicrobial activities of *O. glandulosum Desf.* essential oil and methanolic extract against microorganisms were studied in the present work and their potency was qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameter, and MIC values. The results are given in Table 2.

According to the results given in Table 2, *O. glandulosum Desf.* essential oil displayed a great in vitro potential of antimicrobial activitiy, however the methanolic extract showed no antimicrobial activity. In disc diffusion assay and Micro-well dilution assay, Gram negative strains were extremely sensitive to the 5 µl of essential oil with IZ between 43.66 ± 1.44 (mm) and 59 ± 2.64 (mm), also MIC values ranged from 0.78 (mg/ml) to 1.56 (mg/ml) ; Gram positif strains have also been extremely sensitive: Enterococcus faecalis ATCC 29212 and Staphylococcus aureus ATCC25923 showed a IZ at 51.83 ± 2.46 and 51.83 ± 2.56 respectively, MIC/MIB values were at 0.78 mg/ml. Staphylococcus aureus have been the most sensitive strain with IZ = 63.33 ± 3.05.

Table 3: Free radical scavenging capacities of *Origanum vulgare L.* essential oil, extract and Vitamin C measured in DPPH assay

Sample	IC50 (µg/ml)	AAI
Essential oil	461,62	0,17
Methanolic extract	25,59	3,12
Vitamin C	6,35	12,6

On the other hand Pseudomonas aeruginosa ATCC 27853 was sensitive (IZ=15 ± 1.73 mm and MIC= 6.25 mg/ml) but Pseudomonas aeruginosa were less sensitive to oil with IZ at 8.33 ± 1.15 (mm) and MIC value >50 (mg/ml).

However, all strains seems to be not sensitive to methanolic extract, except Staphylococcus aureus which was moderately sensitive with IZ 12 mm. MBC values were the same as those of MIC for all strains except for Klebsiella pneumoniae ATCC 700603 (MIC 1.56 mg/ml MBC 3.125 mg/ml).

O. glandulosum Desf. essential oil was more active than antibiotic (Gentamycin) on all bacterial strains except for Pseudomonas aeruginosa and Pseudomonas aeruginosa ATCC 27853 which was very resistant to the oil.

These results are significantly higher compared to previous reports of antibacterial investigation on *O. glandulosum* essential oil[25,28] who described IZ ranged between 8-18mm and 8-25 mm respectively.

However, Amrouni et al.[22] essential oil, have been less active against reference strains E.coli and S. aureus than our Oregano essential oil, also, it shows moderate antibacterial activity on Pseudomonas aeruginosa strains (21.6 mm and 1.71 mg/ml). The resistance of Pseudomonas aeruginosa strains is in concordance with other studies[44].

The presence of para-cymene, thymol and carvarol as dominant components in this essential oil and the potential synergistic phenomenon between them might be involved in this great antimicrobial activity.

Thymol is structurally very similar to carvacrol, having the hydroxyl group at a different location on the phenolic ring. Both substances appear to make the cell membrane permeable[45]. Thymol has been previously described as able to interrupt the bacterial membrane, by affecting both the pH gradient and the electron flow across the membrane[45], and it may justify the highest antimicrobial activity of the essential oil.

It was shown that hydroxyl group gets inserted in cytoplasmic membrane, changes the membrane physical and chemical properties and affects both lipid ordering and stability of bilayer, inducing an increase of proton passive flux across the membrane[46].

Despite the p-cymene was an ineffective antimicrobial agent lonely, but combined with carvacrol has led to a synergistic activity resulted by swelling bacterial cell membranes to a greater extent than carvacrol does. By this mechanism p-cymene probably enables carvacrol to be more easily transported across the cytoplasmic membrane so that a synergistic effect is achieved when the two are used together[47].

Antioxidant activity

Amount of total phenolic compounds

The amounts of total phenolics in the extract was determined spectrophotometrically according to the Folin–Ciocalteu procedure and calculated as gallic acid equivalents.

Folin–Ciocalteu reagent consists of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids. These reagent oxidises phenolates resulted in the production of complex molybdenum-tungsten blue which can be detected spectrophotometrically at 725 nm.

Curve equation is: *y (absorbance) = 0.0004 µg gallic acid + 0.1203;* R^2*= 0.9966*

The absorbance value of the methanol extract solution reacted with Folin–Ciocalteu reagent was inserted in the above equation and the total amount of phenolic compound was calculated. The amount of total phenols in the plant methanolic extract was very high and estimated as 526.75 µg GAE/mg.

The results indicated superior total phenolic compounds for methanolic extract compared to Barros et al.[48] who measured high concentration of total phenols (369.18 mg GAE/g extract) in methanol extract of *Origanum vulgare* subsp. *virens.* Sahin et al.[42] also reported a concentration of 220 µg/mg dry extract of *Origanum vulgare ssp. vulgare.*

DPPH radicals scavenging capacity assay

Antioxidant activities of the essential oil and the methanolic extract from *O. glandulosum* have been evaluated by DPPH radical scavenging test.

It is well known that when a solution of DPPH is mixed with that of a substance containing antioxidants, the stable free radical DPPH (deep violet color) is converted to 1,1-diphenyl-2-picryl hydrazine with rise to no color[49].

Free radical scavenging properties of the essential oil and the methanolic extract are presented in Table 3. The activity was expressed in the form of IC50 values. Lower IC50 value indicates higher antioxidant activity.

The methanolic extract showed a very strong scavenging ability on DPPH radicals with IC50 = 25.59 µg/ml and AAI (antioxidant activity index) of 3.12.

On the other hand, the essential oil present poor antioxidant activity with IC50 = 461.62 µg/ml and AAI 0.17. However, DPPH scavenging abilitiy of both methanolic extract and the oil is low compared to reference compound, Vitamin C (IC50 = 6.35 µg/ml and AAI = 12.6).

Previous papers, also, confirmed high radical scavenging ability of different oregano extracts. Sahin et al.[42] observed similar order in their study (synthetic antioxidant > methanolic extract > essential oil), with a strong methanolic extract free radical scavenging activity providing IC50 at 9,9µg/ml. In the free radical scavenging activity, superiority of the methanol extract could be attributed to the presence of phenolics as they comprise 52.67% of the extract.

Compared to prior reports, *O. glandulosum* essential oil, in our study, develop a less DPPH radical scavenging activity. Mechergui et al.[20] have unregistered an essential

oil IC50 at 105.29 mg/L and 142. 86 mg/L. This difference depends on chemical composition of essential oils.

The antioxidant properties of the chemical components of essential oils have shown that monoterpenes, such as para-cymene, have a significant antioxidant effect[50].

According to these results, there is a relationship between total phenol contents and antioxidant activity. Phenolic compounds, biologically active components, are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step[47]. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups[51].

DPPH assay on TLC

In order to visualize qualitatively the radical scavenging capacity of the essential oil and extract, they were subjected to silica gel TLC plates. With DPPH reagent, one big yellow spot appeared after spraying the TLC trace of both essential oil and methanolic extract. Their intensity and reaction speed were also various, suggesting that spots may contain individually different characteristics some of which have a fast radicals scavenging capacity, reducing the DPPH radical very rapidly, while others have a slower RSC, taking a longer time to react[52].

According to color intensities, *Origanum vulgare* methanolic extract has more important radicals scavenging capacity comparing to essential oil. And these results are in agreement with the results by DPPH spectrophotometric assay.

CONCLUSION

The results of this work have shown that *O. vulgare* possess compounds with antimicrobial and antioxidant properties. Essential oil shows a great antibacterial activity against reference clinical strains, and also possesses a moderate antioxidant activity. Moreover, methanolic extract exhibits no antibacterial activity but a high antioxidant activity.

These findings suggest the possibility of using the *O. vulgare* essential oil and methanolic extract as natural alternative to traditional food preservatives and thus enhance food safety and shelf life, and as potential source of antibacterial ingredients in pharmaceutical industry and natural therapies of infectious diseases in human, and controlling of plant disease.

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