Chemical content, antibacterial and antioxidant properties of essential oil extract from Tunisian *Origanum majorana* L. cultivated under saline condition

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Abstract: Essential oils of marjoram were extracted from plants, growing under non-saline and saline condition (75mM NaCl). Their antioxidant and antibaterial activity againt six bacteria (*Enterococcus faecalis*, *Escherichia coli*, *Salmonella enteritidis, Listeria ivanovii*, *Listeria inocula*, and *Listeria monocytogenes)* were assessed. Result showed that, (i) independently of salt treatment, marjoram essential oils inhibited the growth of most of the bacteria but in degrees. The least susceptible one was *Enterococcus faecalis*. (ii) Gram negative bacteria seemed more sensitive to treated essential oils than Gram positive ones. (iii) Compared to synthetic antibiotics, marjoram essential oils were more effective against *E. coli*, *L. innocua* and *S. enteridis*. This activity was due to their high antioxidant activity. Thus, essential oils of marjoram may be an alternative source of natural antibacterial and antioxidant agents.

Keywords: *Origanum majorana* L, salinity, essential oil, antibacterial activity, DPPH

INTRODUCTION

Essential oils extracted from medicinal and aromatic plants are probably suitable as antimicrobial agents. Their utility has been known since long time (Holley and Patel, 2005). Nowadays, studies focused on the use of essential oil having antimicrobial activity on food products (Bajpai *et al*., 2009), as they are natural compounds and they are important not only for food preservation but also for the control of human and plant microbial diseases (Bratta *et al*., 1998). Many *Origanum* species are the subject of the application in various commercial preparations, such as antimicrobials and antioxidants based on study of the chemical composition and antimicrobial properties of their essential oils (Baydar *et al*., 2004). Among them, several studies were known for antibacterial activities such as those of Ben Hamida *et al*. (2001) and Ramos *et al*. (2011); the first authors found, by agar diffusion and broth micro dilution methods, that *Escherichia coli* and *Salmonella enteritidis* were the susceptible bacteria. For Ramos *et al*. (2011), the susceptible one's are *Enterococcus faecalis*, *Escherichia coli.*

In Tunisia, salinity is the major problem for crops. It affected about 10% of the total land area; with the increasing climate aridity, crops were more exposed to salinity (Hachicha *et al*., 1994). Based in previous study, *Origanum majorana* essential oil component, showed a decrease at 100mM NaCl (Bâatour *et al*., 2012), but it could tolerate a moderate concentration (75mM) (Bâatour *et al*., 2010).

What about its antibacterial proprieties? Is there any difference between antibacterial activities of essential oils extract from plants growing under non-saline and saline condition (75mM NaCl)? Thus, we aimed to i) evaluated antibacterial activities of marjoram essential oils (treated and non treated) against bacteria. There are several reports for antibiotics resistance of bacteria to available antibiotics. Bimolecules originated from plant appear to be one of the alternatives for the control of these antibiotic resistant. To respond to this question, we attempt to compare the result with synthetic antibiotics and we finish with the study of antioxidant properties of marjoram essential oil.

MATERIALS AND METHODS

Plant growth conditions (Baatour et al., 2012)
Show paper of Baatour *et al*., (2012).

Essential oil extract, identification and quantification (Baatour et al., 2012)
Show paper of Baatour *et al*., (2012).

GC-FID
Show paper of Baatour *et al*., (2012).

GC-MS
Show paper of Baatour *et al*., (2012).

Antimicrobial assays
Tested and control strains
Essential oil's antibacterial activity was assayed against some bacteria acquired from the American Type Culture

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Collection (ATCC): *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC43265, *Salmonella enteritidis* ATCC 14028, *Enterococcus faecalis* ATCC29212 and clinical isolates from our laboratory: *Listeria innocua* and *Listeria ivanovii.*

Control bacteria were recovered from conserved culture at -80°C. *Listeria* strains were cultured on PALCAM agar, *Salmonella enteritidis* and *Escherichia coli* ATCC isolates on MacKonkey Agar, whereas *Enterococcus faecalis* ATCC was isolated on Slanetz Bartley Agar. Antibacterial tests should be made from young cultures of 18 to 24h in exponential growth phase. From the cultures of 18h, 3 to 5 identical colonies well isolated. They are placed in 5ml of sterile saline. The suspension is standardized to 10^6 colony-forming units CFU.ml^{-1} using a spectrophotometer at a wavelength of 620nm.

Antibacterial activity assay

Antimicrobial activity of essential oils was tested by the agar diffusion technique, according (Rota *et al*., 2004). Briefly, bacterial suspensions was adjusted to 10^7CFU/mL and spread in Mueller Hinton agar (Bio-Rad) using sterile paper discs (Whattman 1; 7mm Ø). They were placed on the surface of Petri dishes and impregnated with 5 µl of essential oil in presence or absence of salt. Petri dishes were then closed and allowed to diffuse essential oil at ambient temperature for 30min. Then, they were incubated overnight at 37°C.

Inhibition zone is characterized by a free zone colonies, or "disk diameter» measured (in mm) and indicated the sensitivity of bacteria tested The degree of measurement was the following (disk diameter included): Resistant (-) = R; diameter ≤8mm; Sensitive (+) = S: diameter between 9 to14 mm; Very sensitive (++): diameter between 15 to 19 mm.

Minimum inhibitory concentration

Inhibitory activities of *Origanum* essential oils (EOs) were determined by the tube dilution method described by Magiatis *et al*. (1999). Essential oils were diluted with tween 80 to have concentrations ranging from 0.25µg/ml to 50µg/ml. The MIC was defined as the lowest concentration of essential oil capable of inhibiting bacterial growth and measurement after 24h and 48h of incubation. The evaluation of MIC was carried out in triplicate. The MIC is the lowest concentration of essential oil capable of inhibiting bacterial growth

Determination of antibiotic pattern.

Bauer *et al*. (1966), tested the antibiotic sensitivity test using standard antibiotics (Ampicillin, Streptomycin, Tetracycline, Gentamycin and Oxacilline). The measurement of inhibition zone of the diameter of the disc (mm) was determined after incubation plates during 24h at 37°C.

Free radical scavenging activity (DPPH)

DPPH of essential oils extract from treated and control marjoram plants, were determined Based to Takao *et al*. (1994). DPPH radical inhibition percentage by the sample was determined using BHT and ascorbic acid as references.

Inhibition % = $(A_{C0} - A_{CS}) / A_{C0}) \times 100$.

AC0: Absorbance of the control, ACS: Absorbance of the sample

The experiment was repeated in triplicates.

$IC_{50:}$ Concentration of essential oil produced a decrease of concentration of DPPH radicals by 50 % were determined from values of percentage of scavenging of DPPH radicals.

The antioxidant activity index (AAI), was calculated according to Scherer and Godoy (2009), as follows: AAI = DPPH concentration in reaction mixture µg mL^{-1}/ CE_{50} µg mL^{-1}

The samples were classified as below: poor antioxidant activity (AAI<0.5), moderate (0.5<AAI<1.0), strong (1.0 <AAI<2.0) and very strong (AAI>2.0) (Scherer and Godoy, 2009).

AAI=DPPH concentration in reaction mixture µg mL^{-1}/ CE_{50} µg mL^{-1}.

Antioxidant activity

Statistical analysis (Baatour et al., 2012)

Results are the means of 3 replicates. Data were subjected to one-way analysis of variance (ANOVA) as variability factor and mean comparison with Duncan post hoc test (Statistica). Means followed by different letters are significantly different at $P \leq 0.005$.

RESULT

Essential oil content

Chemical composition of the Tunisian marjoram essential oil was determined by Bâatour *et al*. (2011). However, its content was evaluated, for the first time, in table 1. Chromatographic analysis allowed the identification of 38 compounds. At control treatment, the major essential oil components were, trans-sabinene hydrate (11.44µg/g DW), followed by terpinen-4-ol (7.018µg/g DW). In contrast, the major ones in essential oil extracted from treated plants (75mM NaCl) were, sabinene (7.723µg/g DW), cis-sabinene hydrate (4.857µg/g DW) and terpinen-4-ol (2.861µg/g DW). According to our result, salinity had a significant effect on the content of essential oil constituents. Since, the major components (trans-sabinene hydrate and terpinen-4-ol) decreased significantly in the presence of 75mM NaCl (table 1). In fact, they decreased respectively about 57% and 59%. But sabinene hydrate and cis sabinene contents increased about 7 and 4 times respectively with salt treatment. Thus, chemotypes in salt conditions became sabinene/ cis sabinene hydrate instead of trans sabinene hydrate/terpinene-4-ol (table 1). Under 75mM NaCl, terpinene-4-ol content decreased about 3 times compared to control, but sabinene hydrate and cis

Table 1: ANOVA analysis and essential oil content µg/g DW of dry sample Tunisian *Origanum majorana* areal part cultured under NaCl 75mM by GC-MS.

No.	Components	RI[a]	RI[b]	0 mM salt	75mM Salt
				Essential oil yield (%)	
				0.118±0.024[a]	0.071±0.01[b]
				Content (µg/ g DW)	
1	Tricyclene	927	1014	0.835±0.18[a]	0.254±0.02 [b]
2	α pinene	931	1035	0.193±0.01 [a]	Nd
3	Sabinene	976	1132	1.546±0.12 [b]	7.723±0.23 [a]
4	Δ-3-carene	1011	1159	0.138±0.03[a]	0.234± 0.10[a]
5	Myrcene	991	1174	Nd	0.073±0.18 [a]
6	α –phellandrene	1006	1176	0.117±0.11[b]	0.193±0.02[a]
7	Limonene	1030	1203	0.094±0.03[a]	0.059±0.01[a]
8	1.8 cineole	1033	1213	Nd	0.053±0.04 [a]
9	γ-terpinene	1062	1266	0.552±0.01[a]	0.424±0.13[a]
10	Terpinolene	1088	1290	0.123±0.02 [a]	0.091±0.00[b]
12	cis-p-menth-2 -1-ol	1129	1562	0.083±0.02[a]	nd
13	trans-p-menth-2 -1-ol	1146	1586	2.360±0.11[a]	0.965±0.06[b]
14	Linalool	1098	1553	0.070±0.20[a]	0.051±0.28 [a]
15	cis sabinene hydrate	1082	1556	1.651±0.18[b]	4.857±0.13[a]
16	trans sabinene hydrate	1053	1474	11.444±1.34[a]	0.309±0.02[b]
17	Linalyl d acetate	1257	1556	0.745±0.18[a]	0.072±0.01[b]
18	bornyl d'acetate	1295	1597	0.075±0.02[a]	nd
19	Carvone	1245	1598	0.014±0.02[b]	0.183±0.04[a]
20	β-elemene	1391	1601	0.364±0.18 [a]	0.177±0.18 [b]
21	terpinene 4-ol	1176	1611	7.018±1.17[a]	2.861±0.03[b]
22	β caryophyllene	1419	1612	0.123±0.05[a]	0.053±0.08[b]
23	α-terpineol	1189	1709	0.057±0.04[a]	0.029±0.01[b]
24	Bicyclogermacreme	1494	1755	1.350±0.12[a]	0.575±0.18[b]
25	neryl acetate	1385	1733	0.447±0.16[a]	0.160±0.04[b]
26	geranyl acetate	1383	1765	0.061±0.05[a]	0.028±0.23[a]
27	Ni	Nd	Nd	Nd	0.077±0.05[a]
28	cis piperitone	1228	1797	0.092±0.05[a]	0.043±0.05[a]
29	Phenol	-	-	0.067±0.18 [a]	0.037±0.03[a]
Classes	Monoterpene hydrocarbons			3.963±0.11[b]	9.168±1.18[a]
	Monoterpenes alcohols			22.750±1.45[a]	9.163±0.28[b]
	Sesquiterpenes hydrocarbons			1.473±0.08[a]	0.628±0.17[b]
	Cetones			0.106±0.01[a]	0.225±0.12[a]
	Esters			0.507±0.03[a]	0.189±0.02[b]

Volatile compound proportions were calculated from the chromatograms obtained on the HP Innowax column. ND non detected. NI non-identified. Values (means of three replicates ± SD) with different superscripts (a–b) are significantly different at P > 0.05. Note: DW: Dry weight

sabinene content increased, that's why we observed more activity of EO in presence than in absence of salt (table 1).

Antibacterial activity of essential oil

An *in vitro* antibacterial assay of *O. majorana* essential oils (Eos) activity, using disc diffusion technique (table 2), showed that Eos exhibited potent inhibitory effect at 5 µl, against all the six tested bacteria. This effect was independently to salt treatment (table 2). With regard to their inhibition diameter zones (16; 18mm and 12; 15 mm, respectively in absence and presence of 75mM NaCl), *E. coli* ATCC25922 and *S. enteridis* were found to be the most inhibited bacteria. *L. innocua, L. ivanovii and Listeria monocytogenes* ATCC 43256 were moderately inhibited. Their inhibition diameter zones ranging respectively, from 8 to 13mm (table 2). In contrast, *E. faecalis* was not inhibited by both types of EO (table 2). Attending to this, an increase in activity was exhibited by EO salt treated, except of control one that showed stronger activities against *L. monocytogenes.*

Table 2: Zone of growth inhibition (mm) of marjoram essential control and treated oil against tested bacteria

			NaCl (mM)		
			0 mM	75 mM	
Microorganisms	Gram (type)	Zone of inhibition (mm)	Results	Zone of inhibition (mm)	Results
L. monocytogenes	+	11±0.02[a]	+	8±0.04[b]	-
L. innocua	+	10±0.12[b]	+	13.9±0.02[a]	+
L. ivanovii	+	12±0.11[b]	+	10±0.12[a]	+
S. enteritidis	-	12±0.05[b]	+	15±0.07[a]	+
E. coli	-	16±0.03[a]	++	18±0.02[b]	+
E. faecalis	+	8±0.04[a]	-	8±0.05[a]	-
P. Larvae	+	7±0.01[b]	+	7±0.11[0]	+
S. aureus	+	9±0.01[b]	+	14±0.11[0]	+

Resistant (-)=R; diameter ≤8mm; Sensitive (+)=S: diameter between 9 to14mm; Very sensitive (++) : diameter between 15 to 19mm. All the data collected for each assay are the averages of three determinations. Values (means of three replicates ± SD) with different superscripts (a–b) are significantly different at P>0.05.

Table 3: Antibiotics susceptibility testing of bacteria

Test bacteria	Antibiotic	Sensibilite/resistance
E. coli	Amoxicilline	R
S. enteritidis	Amoxicilline	I
L. innocua	Tétracycline	R
L ivanovii	Tétracycline	S
L monocytogenes	Tétracycline	S
E faecalis	Gentamicine	S
P Larvae	Tétracycline	S
S aureus	Oxacilline	S

Susceptible (S); Intermediate (I); Resistant (R)

Minimum inhibitory concentrations (MICs)

Following disc diffusion methods and on the basis of the diameter of the inhibition zones formed, four bacterial species were selected. More precise data on the antimicrobial properties were obtained through the determination of the minimum inhibitory concentration (MIC). Thus, from table 3, it can be observed that all the microorganisms tested were susceptible to the marjoram essential oil action, with a range of MIC values from 2.5 µg/ml to 50. µg/ml. Oil displayed remarkable antibacterial effect as minimum inhibitory concentration against two Gram negative bacteria such as *E. coli* ATCC25922 and *S. enteridis*, with MIC values of 5 to 2.5µg/ml and 10 to 5 µg/ml, respectively in absence and presence of salt. The oil also exhibited moderate antibacterial effect against *L. innocua* and *L. ivanovii* with MIC values ranging from 10 to 25µg/ml. EOs extracted from *O. majorana* cultivated in salt constraint medium had a remarkable antibacterial effect than cultivated in control one.

Therefore, the MIC value was in accordance with the disc diffusion method. In addition, the inhibition zone of growth of *L. ivanovii, L. innocua* and *L. monocytogenes* was particularly lower than *E. coli* and *S. enteritidis*. Our findings showed the following order in the sensitivity to the essential oils, measured by inhibition zones and determined by MICs values was as follow, *E. coli*> *S. enteridis*> *L. ivanovii*> *L. innocua*> *L. monocytogenes.* The obtained data showed that Gram negative bacteria were more sensitive to the antimicrobial agent than Gram positive ones.

Essential oil antioxidant activity

Essential oil antioxidant activity was evaluated by their ability to scavenging 2,2-diphenyl-1-picrylhydrazyl stable radicals (DPPH) (Alluri *et al*., 2009). In the present study, the DPPH scavenging activity of the samples was compared with reference compound: Ascorbic acid. The antiradical activity was expressed as IC50: the concentration required to cause 50% DPPH inhibition.

Analyzing antioxidant activity at different EOs concentration, we noted that essential oil had the capacity to scavenge DPPH free radicals (table 4) at any concentration. *O. majorana* control oil, retained important DPPH activity up to the concentration of 1.56 µl.ml^{-1}. It was able to scavenge this radical, rather than treated one (table 4) and it showed, significantly lower IC50 values as compared to treated EO (table 5). It's antioxidant activity exhibited, was significantly higher (AI=0.97) compared to that, extracted from treated plants (table 5, 6). *O. majorana* control EO, showed significantly lower IC50 values (table 6). When compared to standard antioxidants (BHT and ascorbic acid), *O. majorana* oil shows significantly higher antiradical activity in the absence than in presence of salt (table 6).

Table 4: Effect of salt on Minimum inhibitory concentrations (MICs) MIC of *O. majorana* essential oils

E. coli				*S. enteritidis*					
						NaCl (mM)			
0mM	75mM	0mM				75mM		0mM	
						MIC (µg/ml)			
24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
5±0.03[b]	5±0.03[b]	2.5±0.04[a]	2.5±0.04[a]	10±0.12[b]	10±0.12[b]	5±0.05[a]	5±0.05[a]	25±0.14[a]	25±0.15[a]

S. aureus				*L. ivanovii*				*L. innocua*	
NaCl (mM)									
75mM		0mM		75mM		0mM		75mM	
MIC (µg/ml)									
24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
50±0.01[b]	50±0.01[b]	25±0.02[b]	25±0.02[b]	10±0.04[a]	10±0.03[a]	10±0.11[a]	10±0.12[a]	10±0.2[a]	10±0.23[a]

MICs: Minimum inhibitory concentrations

Table 5: Effect of salt on percentage of scavenging activity of DPPH radicals induced by various concentrations of *O. majorana* essential oils

	Concentration of essential oil in absolute methanol (µl.ml-1)							
	50	25	12.5	6.25	3.13	1.56	0.78	0.39
	Percentage (%) of scavenging activity of O. majorana oil NaCl							
0mM	97.20±0.04[a]	95.65±0.11[a]	93.56±0.11[a]	85.63±0.21[a]	72.53±0.31[a]	52.05±0.87[a]	37.24±0.17[a]	24.73±0.02[a]
75mM	93.00±0.12[b]	92.78±0.21[b]	92.24±0.23[a]	73.26±0.01[b]	59.28±0.24[b]	41.49±0.65[b]	28.93±0.32[b]	19.26±0.61[b]

Values (means of six replicates ± SD) with different superscripts (a–b) are significantly different at P>0.05.

Table 6: Concentrations of *O. majorana* essential oils, which produced decrease of DPPH concentration by 50%

	IC50 (µg.ml^{-1})	IC50 (µg.ml^{-1})		
	O. majorana oils	BHT	Ascorbic acid	AAI
0mM	1.85±0.02[b]	2.82± 0.02[b]	4.79±0.19[b]	0.97±0.01[a]
75mM	2.99±0.07[a]	5.60±0.24[a]	7.48± 1.05[a]	0.60±0.10[b]

Values (means of six replicates ± SD) with different superscripts (a–b) are significantly different at P>0.05. IC50: Concentration required causing 50% DPPH inhibition AAI: Antioxidant activity index.

The assessment of antioxidant activity showed that *O. majorana* control EO exhibited significantly higher antioxidant activity (AI=5.4) compared to EO extracted from treated plants (table 5, 6).

Antibiotics susceptibily of bacteria

Standard antibiotics viz. such as Ampicillin, Streptomycin, Tetracyclin, Gentamycin Oxacillin and Amoxicillin, were tested against the six bacteria chosen. Results were exposed in table 3. According to these results, we can affirm that among *Listeria* species only *L. innocua* was resistant to Tetracyclin. *S. enteritidis* are classified as intermediate to Amoxicillin but *E. faecalis, P. Larvae and S. aureus* were sensitive to Gentamycin, Tetracyclin and Oxacillin. We can conclude that, *Origanum* essential oil could be a good natural alternative against previous bacteria

DISCUSSION

Chemical percentage (%) of the Tunisian marjoram essential oil was determined by Bâatour *et al*., (2011). However, the first analysis of its content based on µg g^{-1} DW was evaluated in table 1. As regards the essential oil volatile profile, chromatographic analysis allowed the identification of 29 components. Control EO major compounds, were trans-sabinene hydrate (11.444 µg/g DW), followed by terpinen-4-ol (7.018µg/g DW). In contrast, the major ones in EO extracted from treated plants (75mM NaCl), were sabinene (7.723µg/g DW), cis-sabinene hydrate (4.857µg/g DW) and terpinen-4-ol (2.861µg/g DW). According to our result, salinity had a significant effect on the content of EO constituents. Since, the major components trans-sabinene hydrate and terpinen-4-ol decreased significantly in the presence of 75 mM NaCl (table 1). They decreased respectively about

57% and 59%. But sabinene hydrate and cis sabinene contents increased about 7 and 4 times respectively with salt treatment. Thus, chemotypes in salt conditions became sabinene/ cis sabinene hydrate instead of trans sabinene hydrate/terpinene-4-ol (table 1). Under 75mM NaCl, terpinene-4-ol content decreased about 3 times compared to control, but sabinene hydrate and cis sabinene content increased.

It was recognized that the antibacterial activity of essential oils and their action were directly influenced by the nature and proportion of their constituents (Guinoiseau *et al*., 2010). and that the major compounds were often responsible for the antibacterial activity (Kalemba and Kunicka, 2003). According to foregoing, we could consider that in control condition; bioactivity of *Origanum* EO could be assumed to trans- sabinene hydrate, followed by terpinene 4-ol and sabinene. In salt conditions, bioactivity could be assumed to sabinene followed by cis- sabinene hydrate and terpinene 4-ol. But if we reffered to Ramos *et al*. (2011) study, they demonstrate that fraction of EOS rich in cis-sabinene hydrate was more active, on bacterial growth inhibition, than the one rich in terpinen-4-ol; we could conclude that EO, in salt condition would be more active than in control one. But, in fact it was not the case; because we found large inhibition zones and low MIC values, in presence and absence of salt. On the basis of the results above, the antibacterial activity is probably related to their high monoterpenes content such as sabinene and monoterpenes alcohols like cis-sabinene hydrate and trans-sabinene hydrate.

Our results showed that Gram-negative bacteria were more sensitive to the essential oil of *Origanum* than Gram positive ones. This finding was in agreement with those of Burt (2004), Bussata *et al*. (2008) and Ben Sghaier (2007), who tested many EOs of (*Satureja Horthensis*, *Mentha piperita*, *Lavandula angustifolia)* against Gram negative and Gram-positive bacteria. How can we explain this difference in sensitivity? Bagamboula *et al*. (2004) reported that, the greater resistance of Gram-negative bacteria, to essential oils, has been attributed in part to the great complexity of the double membrane-containing cell envelope of these microorganisms; in contrast to the single membrane structures of Gram-positive bacteria. According to Levic *et al*. (2011), it appears that the difference in antibacterial activities may be related to the concentration and nature of contents, the functional groups, the structural configuration of the components and their possible synergistic interaction. For us, these differences found between essential oils could due to ecological factors, plant growth factors, basic test method, type of emulsifier used, and incubation time.

Data above demonstrate the variability in resistance among *Listeria* species. In fact the human pathogen *L. monocytogenes* and *L. ivanovii* were susceptible to Tetracycline except of *L innocua* were resistant.

Furthermore, the scientists discovered that there were "differences in resistance among *Listeria* spp.," with *L. innocua* showing the most resistance to the antibiotics and *L. monocytogenes* showing the least one (Li and Sherwood, 2004). The significance of this fact is that the high antimicrobial resistance displayed in *L. innocua* has the potential to transfer resistance to the low-resistance *L. monocytogenes*. This could be detrimental because high-resistance *L. monocytogenes* poses a more dangerous threat to public health (Li and Sherwood, 2004).

Salmonella Enteritidis are classified as intermediate to Amoxicilline. *E faecalis, P Larvae and S aureus* isolates showed sensitivity respectively to Gentamicine, Tetracycline and Oxacilline Actually, 70% of strains were resistant at least to one antibiotic and it may be because of administration of antibiotic in industry and medicine.

The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metal ion catalysts (Mao *et al*., 2006). It is thus important that for evaluating the effectiveness of antioxidants, several analytical methods and different substrates are used. Exposure of plants to unfavorable environmental conditions such as salinity could increase the production of ROS (Reactive Oxygen Species). To prevent constraints damage and resist to various stress, plant increased levels of antioxidants (Bor *et al*., 2003). In a previous work, according to Bâatour *et al*. (2012), a greater tannin content was observed in Tunisian variety enhance antioxidant activities in marjoram in presence of salt constraint.

The differences in antioxidant activity of EO extracted from control and treated samples could be explained by altered composition of essential oils, which was recorded during the experiment. In order to know which of the chemical constituents of the oil were responsible for antimicrobial activity, further the future experiment was designed to screen constituents of the essential. In fact, the antimicrobial activity may be mainly attributed to the active components in EO, meanwhile, the synergistic effects of these active chemicals with each other and minor constituents of EO should also be taken into consideration for the bioactivity.

CONCLUSION

This study showed that essential oils of marjoram extract from plant grew under control or saline conditions, were effective to inhibit the growth of majority of bacteria tested except *Paenibacillus larvae*. EOs revealed stronger

activities against Gram negative than Gram-positive bacteria. Activities increase with salt constraint. Compared to antibiotics activities, essential oils of marjoram had the same activity against some ones; thus it could be a good and natural alternative.

Additionally, control essential oils revealed the strongest antioxidant activity. Antibacterial and antioxidant activities of these essential oils could be due, in part, to the presence of several compounds, like sabinene, cis-sabinene, trans-sabinene and terpinene-4-ol in their chemical compositions.

More importantly, these can be included in the list of herbal medicines due to their high antimicrobial potential. Hence, essential oils and their components can be recommended for therapeutic purposes and be used as an alternative medicine.

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