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RESEARCH ARTICLE

α-Amylase Inhibitory activities of *Origanum glandulosum*, a North African endemic species

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Abstract

The essential oils, isolated by hydrodistillation from the aerial parts of *Origanum glandulosum* collected at the vegetative stage in Tunisia, were analysed by GC-MS. Antioxidant activities were analysed by radical scavenging activity and ferric reducing antioxidant power assay, and metal chelating effects. α-amylase inhibitory effect of volatiles compounds were screened. A total of 40 constituents representing 95.35% of the total oil were identified. The essential oils were dominated by the oxygenated monoterpenes fraction (66.18% to 78.17%), carvacrol being the main component of all oils (65.01%). The other main components were p-cymene (9%) and γ-terpinene (4.25%). DPPH and FRAP assays showed that leaves of *O. vulagre* have a potent antioxidant activities which was comparable to Trolox. The essential oil was screened for α-amylase inhibitory effect, showed a strong capacity to inhibit the degradation of starch by pancreatic and salivary α-amylase. These results show that *O. glandulosum* essential oil could be considered as a natural antioxidant and antidiabetic agent and may be useful in the pharmaceutical and food industries.

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Introduction

Origanum is one of the economically important plants of the Lamiaceae. This genus is characterised by extensive morphological and chemical diversity. Due to variability in chemical and aroma characteristics, *Origanum* plants are widely used in agriculture, pharmaceutical and cosmetic industries as a culinary herb, flavouring substances of food products, alcoholic and perfumery for their spicy fragrance (Novak et al., 2000; Aligiannis et al., 2001). Dried *Origanum* species are used for the production of essential oil and aromatic water (Dundar et al., 2008) and they are used as stimulants, analgesics, expectorants, sedatives and antiparasitisc in folk medicine, but mostly for gastrointestinal complaints (Başer, 2002). They are also rich in bitter substances (Esen, 2007; Bekhechi et al., 2008). Phytochemicals from *Origanum* species have an increased interest due to their antimicrobial (Khalfi et al., 2008), antifungal, insecticidal, and antioxidant properties (Kouri et al., 2007).

Many medicinal plants have shown antidiabetic activity (Latha et al., 2004). Polyphenols from berry (McDougall and Stewart, 2005) exhibit an inhibitory effect on α-amylase. Rosmarinic acid-containing oregano extracts yielded higher than expected amylase inhibition, suggesting that other phenolic compounds such as carvacrol may contribute to additional amylase inhibitory activity (McCue et al., 2004). Diabetes mellitus (DM) is the most common endocrine disorder known and results in deficient insulin production (type 1 DM) or combined resistance to insulin action and the insulin-secretory response (type 2 DM) (American Diabetes Association, 2005). One of the therapeutic approaches for decreasing post-prandial hyperglycaemia is to prevent absorption of glucose by the inhibition of carbohydrate-hydrolysing enzymes, such as α-amylase (Shobana et al., 2009). Thus, the retardation of the action of α-amylase by inhibitors might be one of the most effective approaches to control type 2

DM. Furthermore, taking into account its present rate of increase, it will be one of the world's commonest diseases and one of the biggest public-health problems (Diamond, 2003).

O. glandulosum Desf., synonymous of *O. vulgare* L. subsp. *glandulosum* (Desf.) Ietswaart, is an endemic species of Algeria and Tunisia, called ‘‘Zaâtar el Mlouk’’ in Arabic (Boulos, 1983), a very popular mixture that is used almost daily in the Middle East as food, additive in salads and spice for pastry, and meat. This plant is used in folk medicine to cure several diseases such as rheumatism, coughs and colds (Mahmoudi, 1990). With its high content of volatile oils, it has been reported that oregano comprises various medicinal benefits such antibacterial (Béjaoui et al., 2013), antifungal (Penalver et al., 2005) and antioxidant properties (Bejaoui et al., 2013).

The objective of this study was to get knowledge on the essential oil composition of Tunisian endemic plants and evaluate their antioxidant capacity and α-amylase inhibitory activity of *O. glandulosum* for a possible valorization of essential oil.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6 tripyridyl-s-triazine), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), $FeCl_3$ $6H_2O$, $FeCl_2$, $4H_2O$, Ferrozine, $FeSO_4$ $7H_2O$, KI and Soluble starch (S-9765) were purchased from Sigma-Aldrich (St. Louis, MO). All solvents and reagents used in this study are higher purity.

2.2. Plant material

The oregano plants used for this study were collected at the vegetative stage from Sidi Nssir Mountain (35°27’N and 9°33’E) located in North East of Tunisia. The site belongs to the sub-humid bioclimatic zone with a rainfall ranging between 600 and 900 mm/year. Botanical identification of this species was carried out by Prof. M. Boussaid, Biologist (National Institute of Applied Science and Technology, Tunisia). Voucher specimens were deposited at the herbarium of INSAT.

2.3. Isolation of essential oils

Oils were obtained by hydrodistillation, using a Clevenger-type apparatus, for 3 h of 30 to 60 g of air-dried leaves of each sample. Oil yields were then estimated on the basis of the dry weight of plant material. Oils were recovered directly, from above the distillate, and stored in dark vials at 4°C.

2.3.1. Essential oils identification

Gas chromatography (GC) analyses were performed using an Agilent 6890 N gas chromatograph equipped with a flame ionisation detector (FID) and an electronic pressure control (EPC) injector. Non-polar HP-5 MS columns (30 m x 0.25 mm, 0.25 μmol film phenyl methyl siloxane) were used. The carrier gas was helium with a flow rate of 1ml/min. The split ratio was 50:1. All analyses were performed using the following temperature ramp: oven kept isothermally at 50 °C for 1 min, increased from 50 to 250°C at the rate of 8°C/min and then kept at 250 °C for 10 min. Injector and detector temperatures were held at 220 and 280 °C, respectively.

The essential oils were analysed by Gas chromatography–mass spectrometry (GC–MS) using a HP 5975C mass spectrometer (Agilent technologies) with electron impact ionization (70 eV). A HP-5MS capillary column (30 m x 250 μm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 μmol film thickness) was used. Oven temperature was programmed to rise from 60 to 220°C at a rate of 4°C/min; transfer line temperature was 230°C. The carrier gas was He with a flow rate of 0.8 ml/min and a split ratio of 50:1. Scan time and mass range were 1 s and 50-550 m/z, respectively (Messaoud et al., 2005).

The identification of oil components was assigned by comparison of their retention indices (RI) relative to (C8-C22) n-alkanes with those of literature or with those of authentic compounds available in the authors’ laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system and other published mass spectra (Adams, 2001). The percentage of components was based on peak area normalization without using correction factors.

2.4. Evaluation of antioxidant activity

The antioxidant activity of oils was assessed using free radical-scavenging activity (RSA), ferric reducing antioxidant power assay (FRAP) systems and metal chelating activity.

2.4.1. Free radical-scavenging activity

The evaluation of the free radical-scavenging activity was based on the measurement of the reducing ability of antioxidants toward the DPPH radical. Various concentrations of the diluted essential oils were mixed with 0.50 ml DPPH (0.2 mmol). The mixture was then shaken and allowed to stand at room temperature in the dark. After 30 min, the decrease in absorbance was measured at 517 nm against a blank (methanol solution) using a UV-vis spectrophotometer. A mixture consisting of 0.50 ml of methanol and 0.25 ml of DPPH solution was used as the control.

The capability to scavenge the DPPH radical was calculated using the following equation: RSA (%) = 100× (A blank - A sample)/ A blank; where A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Trolox was used as the positive control. Three replicates of sample were recorded.

The IC50 value, which represented the concentrations of the essential oil that caused 50% inhibition, was determined by linear regression analysis from the obtained RSC values.

2.4.2. Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was adapted from Gardeli et al. (2008) The FRAP reagent was freshly prepared by mixing TPTZ solution (10 mmol TPTZ in 40 mmol HCl), ferric solution ($FeCl_3.6H_2O$, 20 mmol) and acetate buffer (300 mmol, pH 3.6) in proportions of 1:1:10 (v/v). To perform the assay, 900 µl of FRAP working reagent were mixed with 90 µl distilled water. 30 µl of diluted oil were then added and incubated at 37°C in a water bath for 30 min. Absorbance at 593 nm was determined against distilled water blank. Ferrous sulfate heptahydrate solutions (100-2000 µmol) were used for calibration. The FRAP values were expressed as mmol of Fe^{2+}/g DW. All assays were determined in triplicate.

2.4.3. Metal chelating activity

The chelating of ferrous ions by *O. gladulosum* essential oils was estimated as described by Dinis et al. (1994) Different concentrations of extracts were added to a 0.3 ml $FeCl_2$, $4H_2O$ solution (1 mmol/l) and left for incubation for 5 min. The reaction was initiated by adding 0.3 ml of ferrozine (25 mmol/l). Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+}complex formation was calculated using the formula given bellow:

Metal chelating effect (%) = (A0 - A1)/A0× 100; where A0 is the absorbance of the control, and A1 is the absorbance in the presence of the sample or standard. Analyses were run in triplicates.

2.5. α-Amylase inhibition assay

The samples were tested for amylase inhibition activity by the agar disc diffusion method, according to Cha et al. (2009). Starch hydrolysis assay was performed on plates composed of 1% (w/v) starch dispersed in 1.5% agar. Sterile Whatman No. 1 (ϕ= 8 mm) disc papers were individually placed on agar plates, and then 10 µl of samples (pancreatic α-amylase with or without sample) were applied to the filter paper disc. After incubation at 37°C for 72 h, starch plates were stained by flooding with iodine solution (5 mmol I_2 in 3% KI) for 15 min at room temperature. Iodine was removed from the plates by washing with distilled water. Amylase activity was determined by observing the zone diameter of hydrolyzed areas around the wells.

The percentage of inhibition of α-amylase was calculated using the formula given bellow: Amylase activity (%) = (A0 - A1)/A0× 100; where A0 is the diameter of the negative control, and A1 is the diameter of the sample.

3. Results and discussion

3.1. Yields and Chemical composition of essential oils

The essential oils isolated from *O. glandulosum* were obtained in different yields, according to individuals ranged from 6.84 to 7.48%. Forty of their components were identified, amounting for 90.31 to 98.14% of the total oils from the samples isolated by hydrodistillation. The identified components in oil, and their percentages, are listed in Table 1.

The oxygenated monoterpene fraction was the most representative one in all the oils, ranging from 66.18 to 78.17%. This fraction was dominated by the oxygen-containing compounds, carvacrol being clearly the main component of all oils (62.08 to 74.03%), p-cymene (5.89 to 12.60%) and γ -Terpinene (1.13 to 6.88%) were the second and third main components of all oils. Borneol was present also in a relatively high amount in all oils (1.80 to 4.2%).

These results highlight the great variability of chemical composition of oregano oil. These differences could be due either to ecological and genetic factors. The oil of Tunisian samples collected from the North of Tunisia at Nefza, Krib and Bargou regions at the flowering stage was shown to be rich in thymol (18-31%), γ-terpinene (11-24%) and p-cymene (35-46%) (Mechergui et al., 2010).

The principal constituent of oxygenated monoterpenes is the phenolic compounds. Carvacrol, has a characteristic pungent warm taste and odor and used in several products as an antimicrobial agent (Béjaoui et al., 2013), showing a broad-spectrum of activities against bacteria, yeasts and fungi (Knowles, 2005). It has been believed that the production of carvacrol seems to depend on some external variation such as the soil, climatic conditions, harvesting time and the amount of water to which the herb is exposed. It is also stated in the literature that there is no clear and direct relationship of the influence of environmental conditions on the percentages of carvacrol (Loziene et al., 2005).

Table 1. Mean percentage of volatiles of *Origanum glandulosum* isolated from the aerial parts collected during the vegetative phase.

No	Compound	*RI*[a]	*Sample*								*Average*[b]
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
1	α-thujene	924	0.85	1.18	1.20	1.04	0.80	1.24	0.66	0.92	0.99(0.07)
2	α-pinene	940	0.52	-	0.48	0.65	0.39	0.68	0.41	0.42	0.44(0.07)
3	Camphene	953	0.47	0	0.39	0.44	0.33	0.66	0.29	0.37	0.37(0.07)
4	sabinene	979	-	0.04	0.06	0.04	0.08	0.08	-	0.04	0.04(0.01)
5	β-pinene	983	-	-	-	0.31	-	0	0.25	-	0.07(0.05)
6	Β-myrcene	992	1.24	0.42	0.77	1.42	0.67	0.89	0.93	1.23	0.95(0.12)
7	3-octanol	995	0.13	0.14	0.17	0.12	0.09	0.28	-	0.19	0.14(0.03)
8	α-phellandrene	1006	0.23	0.29	0.21	0.26	0.17	0.21	-	0.23	0.20(0.03)
9	Delta-car-3-ene	1009	0.08	0.10	0.11	0.09	0.11	0.10	0.08	0.08	0.09(0.00)
10	α-Terpinene	1017	1.24	1.60	1.07	1.37	0.84	1.55	1.20	1.15	1.25(0.09)
11	p-Cymene	1026	7.90	8.99	10.76	9.87	5.89	12.60	7.56	8.39	9.00(0.73)
12	Limonene	1030	0.53	0.68	-	-	0.36	-	0.41	0.50	0.31(0.10)
13	Trans-alpha-ocimene	1035	0.11	0.18	0.17	1.39	0.03	0.89	0.92	1.33	0.63(0.20)
14	β-ocimene	1040	-	0.07	-	0.07	-	0.04	0.06	0.08	0.04(0.01)
15	γ-terpinene	1059	5.75	6.88	1.13	6.34	2.06	2.58	4.49	4.75	4.25(0.75)
16	Cis-Sabinene hydrate	1072	0.64	0.68	1.36	0.81	0.76	1.30	0.63	0.67	0.86(0.11)
17	α-terpinolene	1088	0.19	0.26	0.12	0.19	0.10	0.17	0.12	0.18	0.17(0.02)
18	Trans-Sabinene hydrate	1101	0.37	0.39	0.57	-	0.35	0.57	0.34	0.44	0.38(0.06)
19	α-Campholene aldehyde	1125	-	-	-	0.08	0.08	0.18	0.04	0.15	0.07(0.02)
20	Camphor	1141	-	-	0.09	-	-	0.09	-	0.06	0.03(0.02)
21	Borneol	1164	3.72	4.25	3.26	2.38	2.24	4.87	1.80	2.96	3.19(0.37)
22	Terpinen-4-ol	1181	1.03	1.17	0.82	0.95	-	0.77	0.67	0.99	0.80(0.13)
23	Carvone	1240	-	-	-	0.09	-	0.12	0.09	0.11	0.05(0.02)
24	Carvacrol methyl ether	1242	0.03	-	0.12	0.14	-	0.07	0.08	0.08	0.07(0.02)
25	Thymol	1293	0.39	0.29	0.24	0.21	0.07	0.34	0.29	0.27	0.26(0.03)
26	Carvacrol	1302	68.45	62.09	67.64	61.08	74.03	61.20	63.50	62.08	65.01(1.6)
27	Eugenol	1353	-	-	-	0.04	0.06	0.07	-	-	0.02(0.01)
28	Carvacryl acetate	1367	0.73	0.74	0.78	0.52	0.72	0.10	0.46	1.61	0.71(0.15)
29	β-caryophyllene	1421	1.15	1.25	3.04	1.62	1.13	2.35	1.47	2.30	1.79(0.25)
30	Aromadendrene	1437	0.15	0.23	0.11	0.17	0.06	0.12	0.16	0.08	0.14(0.02)
31	α- Humulene	1445	-	-	-	-	-	0.12	0.09	0.11	0.04(0.02)
32	Germacrene D	1477	0.13	0.20	0.12	0.17	0.16	0.13	0.15	0.46	0.19(0.04)

Table 1 (Cont.)

33	α-amorphene	1487	0.15	0.20	0.15	0.22	0.20	0.27	0.20	0.31	0.21(0.02)
34	Bicyclogermacrene	1491	0.34	0.42	0.44	0.38	0.36	0.76	0.35	0.29	0.42(0.05)
35	β-bisabolene	1510	0.84	1.17	0.70	1.64	0.56	1.24	1.49	2.87	1.31(0.26)
36	Spathulenol	1581	0.25	0.25	0.23	0.36	0.12	0.41	0.33	0.17	0.27(0.03)
37	Caryophyllene oxide	1585	0.38	0.43	0.80	0.82	0.39	0.50	0.74	-	0.51(0.10)
38	β-Eudesmol	1643	-	-	-	0.06	-	-	0.05	-	0.01(0.01)
39	δ-Muurolene	1685	-	-	0.07	0.06	-	-	-	-	0.02(0.01)
40	Lanceol	1770	0.15	0.17	0.08	0.33	-	-	0	-	0.09(0.04)
Total identified			**98.14**	**94.76**	**97.26**	**95.73**	**93.21**	**97.55**	**90.31**	**95.87**	**95.35**
Chemical classes											
Monoterpene hydrocarbons			19.11	20.69	16.47	23.48	11.83	21.69	17.38	19.67	**18.79**
Oxygenated monoterpenes			75.36	69.61	74.88	66.18	78.17	69.43	67.86	69.27	**64.35**
Sesquiterpene hydrocarbons			2.76	3.47	4.63	4.26	2.47	4.99	3.91	6.42	**12.72**
Oxygenated sesquiterpenes			0.78	0.85	1.11	1.57	0.51	0.91	1.12	0.17	**1.31**
Others			0.13	0.14	0.17	0.24	0.23	0.53	0.04	0.34	**0.35**

[a)] retention indices *(HP-5MS)*

[a]) retention index in a *hp-innowax* capillary column,

-: not detected,

(): standard deviation.

Table 2. DPPH radical scavenging activity (IC_{50}), reducing power, metal chelating, and α-amylase inhibition activites of *O. glandulosum* essential oils.

	DPPH radical-scavenging $IC_{50}{}^{b}$ (mg/ml)	FRAP (mg/ml)	Metal chelating[c] (mg/g)	α-amylase inhibition (%)		
			Dilutions:	1/10	1/20	1/40
Eessential oil	0.625±0.09	0.89±0.03	38.11±0.8	90	80	75
Trolox	0.5±0.02					

[a]: Results are reported as mean ± standard deviation of three replicates (95% confidence)); [b]: mg trolox equivalents/ml; [c]: mg eq EDTA/g DM.

3.2. Antioxidant and Metal chelating activities

The essential oils showed a substantial antioxidant activity (Table 2), although the two essays (DPPH and FRAP) yielded different values of antioxidant activity. It exerted an antioxidant activity which was comparable to that Trolox as shown by DPPH assay. However, the DPPH assays shown that Trolox exhibited similar antioxidant activity (IC50 = 0.5 mg/ml) than the essential oils (IC50 = 0.625) (Table 2). The plant extracts of *Origanum* gave ferrous equivalent values of essential oils (0.89 mg/ml).

A relatively high metal scavenging activities were detected in the essential oils (38.11 mg eq EDTA/g DM) (Table 2).

The *O. vulgare* essential oil analyzed in this research showed potent antioxidant activities. According to GC-MS analysis, the species contains high levels of phenolic compounds (carvacrol). The antioxidant activity of *O. vulgare* essential oil is thus likely thus related to carvacrol (Béjaoui et al., 2013). In several study, carvacrol, was found to be main antioxidant constituents of the oils isolated from several *Origanum* species (Puertes-Mejia et al., 2002).

3.3. α-Amylase inhibition assay

The inhibition of α-amylase activity is considered to be an effective strategy for the control of diabetes by diminishing the absorption of glucose (Hara and Honda, 1990). The degradation of starch by pancreatic α-amylase was inhibited by oils of *O. vulgare*. However, the dilutions (1/10, 1/20, and 1/40) displayed high inhibition activity of α-amylase (90, 80 and 75%, respectively) (Table 2). Rosmarinic acid-containing oregano extracts yielded higher than expected amylase inhibition, suggesting that other phenolic compounds such as carvacrol may contribute to additional amylase inhibitory activity (McCue et al., 2004). These results agree with other reports, demonstrating the ability of phenolic substances to interact with and/or inhibit proteins/enzymes (Rhon et al., 2002).

4. Conclusion

Our analysis on Tunisian *Origanum* oil extracted from air dried samples has led to the identification of 40 compounds. Essential oils showed high antioxidant activities. These results indicate that the oregano essential oils could be in use as potential resource of natural antioxidants for food industry so that it is interesting to examine its application as additive in some food products. There is a great interest in natural sources of antioxidants to prevent oxidative degradation of foods and to minimize oxidative damage to living cells.

Natural α-amylase inhibitors from *O. glandulosum* offer a therapeutic approach to the treatment of hyperglycemia by decreasing glucose release from starch and may have potential for use in the treatment of diabetes mellitus and obesity.

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References

Adams, R.P. (2001): Software library called identification of essential oil components by Gas chromatography/quadrupole mass spectroscopy. Illinois: Allured Publishing.

Aligiannis, N., Kalpoutzakis, E., Mitaku, S. and Chinou, I.B. (2001): Composition and antimicrobial activity of the essential oils of two *Origanum* species. J. Agric. Food Chem., 49: 4168-4170.

American Diabetes Association, (2005): Diagnosis and classification of diabetes mellitus. Diabete Care, 28: 37-42.

Başer, K.H.C. (2002): The Turkish *Origanum* species. In: Kintzios SE (eds) *Origanum,* the genera and *Origanum* and *Lippia*: Taylor and Francis. London and New York, pp. 109-126.

Baytop, T. (1999): Therapy with medicinal plants in Turkey: today and in future. Istanbul University Press, Istanbul, pp. 166-167

Béjaoui, A., Boulila, A. and Boussaid, M. (2013): Chemical composition and biological activities of essential oils and solvent extracts of *Origanum vulgare* subsp. *glandulosum* Desf. from Tunisia. J. Med. Plants Res., 7(32): 2429-2435.

Béjaoui, A., Chaabane, H., Jemli, M., Boulila, A. and Boussaid, M. (2013): Essential oil composition and antibacterial activity of *Origanum vulgare* subsp. *glandulosum* Desf. at different phenological stages. J. Med. Food, 16(12): 1115-1120.

Bekhechi, C., Atik-Bekkara, F. and Abdelouahid, D.E. (2008): Composition et activité antibactérienne des huiles essentielles d'*Origanum glandulosum* d'Algérie. Phytothérapie, 6: 153-159.

Boulos, L. (1983): Medicinal Plants of North Africa. Reference Publication: Algonac, M.I.

Cha, W.S., Ju, I.S., Yun, D.H., Chun, S.S., Kim, J.H. and Cho, Y.J. (2009): Biological activity of extracts from cherry sage (*Salvia officinalis* L.). Korean J. Life Sci., 19: 390-396.

Diamond, J. (2003): The double puzzle of diabetes. Nature, 423: 599-602.

Dinis, T.C., Madeira, V.M. and Almeida, L.M. (1994): Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochem. Biophys., 315: 161-169.

Dundar, E., Gurlek, O.E., Isiksoy, S., Kurkcuoglu, M., Başer, K.H.C. and Bal, C. (2008): The effects of intra-rectal and intra-peritoneal application of *Origanum onites* L. essential oil on 2,4,6-trinitrobenzenesulfonic acid-induced colitis in the rat. Exp. Toxic. Pathol., 59: 399-408.

Esen, G., Azaz, A.D., Kurkcuoglu, M., Baser, K.H.C. and Tinnaz, A. (2007): Essential oil and antimicrobial activity of wild and cultivated *Origanum vulgare* L. subsp. *hirtum* (Link) letswaart from the Marmara region, Turkey. Flav. Fragr. J., 22(5): 371-376.

Gardeli, C., Vassiliki, P., Athanasios, M., Kibouris, T. and Komaitis, M. (2008): Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis*: Evaluation of antioxidant capacity of methanolic extracts. Food Chem., 107: 1120-1130.

Hara, Y. Honda, M. (1990): The inhibition of α-amylase by tea polyphenols. Agric. Biol. Chem., 54: 1939-1945.

Khalfi, O., Sahraoui, N., Bentahar, F. and Boutekedjiret, C. (2008): Chemical composition and insecticidal properties of *Origanum glandulosum* (Desf.) essential oil from Algeria. J. Sci. Food Agric., 88: 1562-1566.

Knowles, J.R., Roller, S., Murray, D.B. and Naidu, A.S. (2005): Antimicrobial action of carvacrol at different stages of dual species biofilm development by *Staphylococcus aureus* and *Salmonella enteric, Serovar Typhimurium*. App. Envir. Microb., 71(2): 797-803.

Kouri, G., Tsimogiannis, D., Bardouki, H. and Oreopoulou, V. (2007): Extraction and analysis of antioxidant components from *Origanum dictamnus*. Innov. Food Sci. Emerg. Technol., 8: 155-162.

Latha, M., Pari, L., Sitasawad, S. and Bhonde, R. (2004): *Scoparia dulcis*, a traditional antidiabetic plant, protects against streptozotocin induced oxidative stress and apoptosis in vitro and in vivo. J. Bioch. Mol. Toxic., 18: 261-272.

Loziene, K. and Venskutonis, P.R. (2005): Influence of environmental and genetic factors on the stability of essential oil composition of *Thymus pulegioides*. Bioch. Sys. Ecol., 33 : 517-525.

Mahmoudi, Y. (1990) : La thérapeutique par les plantes communes en Algérie : Palais du livre, Blida.

Mastelic, J., Jerkovic, I., Blazevic, I., Poljak-Blazi, M., Borovic, S., Ivancic-Bace, I., Smrecki, V., Zarkovic, N., Brcic-Kostic, K., Vikic-Topic, D. and Müller, N. (2008): Comparative study on the antioxidant and biological activities of carvacrol, thymol, and eugenol derivatives. J. Agric. Food Chem., 56: 3989-3996.

McCue, P., Lin, Y.T., Labbe, R.G. and Shetty, K. (2004): Sprouting and solid-state bioprocessing by dietary fungi increase the in vitro antibacterial activity of aqueous soybean extracts against Helicobacter pylori. Food Biotech., 18: 229-249.

McCue, P., Lin, Y.T., Labbe, R.G. and Shetty, K. (2004): Sprouting and solid-state bioprocessing by dietary fungi increase the in vitro antibacterial activity of aqueous soybean extracts against *Helicobacter pylori.* Food Biotech., 18: 229-249.

McDougall, G.J. and Stewart, D. (2005): The inhibitory effects of berry polyphenols on digestive enzymes. Biofactors, 23: 189-195.

Mechergu,i K., Coelho, J.A., Serra, M.C., Lamine, S.B., Boukhchina, S. and Khouja, M.L. (2010): Essential oils of *Origanum vulgare* L. subsp. *glandulosum* (Desf.) Ietswaart from Tunisia: chemical composition and antioxidant activity. J. Sci. Food Agric., 90: 1745-1749.

Messaoud, C., Zaouali, Y., Bensalah, A., Khouidja, M.L. and Boussaid, M. (2005): *Myrtus communis* in Tunisia, Variability of the essential oil composition in natural populations. Flav. Fragr. J., 20: 577-588.

Novak, J., Christina, B., Langbehn, B., Pank, F., Skoula, M., Gotsiou, Y. and Franz, C.M. (2000): Ratios of cis- and trans-sabinene hydrate in *Origanum majorana* L. and *Origanum midrophyllum* (Bentham) Vogel. Bioch. Sys. Ecol., 28(7): 697-704.

Penalver, P., Huerta, B., Borge, C., Astorga, R., Romero, R. and Perea, A. (2005): Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. APMIS., 113: 1-6.

Puertes-Mejia, M., Hillebrand, S., Stashenko, E. and Winterhalter, P. (2002): In vitro radical scavenging activity of essential oils of Columbian plants and fractions from oregano (*Origanum vulgare* L.) essential oil. Flav. Fragr. J., 17: 380-384.

Rohn, S., Rawel, H.M. and Kroll, J. (2002): Inhibitory effects of plant phenols on the activity of selected enzymes. J. Agric. Food Chem., 50: 3566-3571.

Shobana, S., Sreerama, Y.N. and Malleshi, N.G. (2009): Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. Food Chem., 115: 1268-1273.