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EXTRACTION AND ANTIOXIDANT ACTIVITIES OF TWO SPECIES *ORIGANUM* PLANT CONTAINING PHENOLIC AND FLAVONOID COMPOUNDS

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ABSTRACT

The antioxidant of ethanolic extract of two species of *Origanum* and essential oil of plant *Origanum vulgare* were investigated and also the total phenolic and flavonoid content measured. The radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Total phenolic and flavonoid contents were estimated by Folin-Ciocalteu and aluminum chloride methods, respectively. According to the results the leaves extracts have very important values for polyphenols (266.86 mg GAE / g and 194.78 mg GAE / g) and high antioxidant activity; DPPH (IC_{50} = 1.37 g / l and IC_{50} = 1.53mg / l) for species *majorana*, and *vulgare* respectively; also the DPPH of essential oil of *Origanum vulgare* was IC_{50} = 15.360 mg/l . This data suggest of these extracts as a natural source of phenolic compounds and antioxidant.

Key words: *Origanum majorana*, *Origanum vulgare*, polyphenol, flavanoid, DPPH.

1. INTRODUCTION

Nowadays, a large number of compounds derived from plants are used in modern medicine and a majority of them are inspired by traditional applications. About 60% of anticancer drugs and 75% of compounds for infectious diseases are either natural products or their derivatives. However, it is estimated that only bioactive molecules of 5 to 15% of more than 250000 plants species on earth have been investigated.

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Marjoram was formerly classified as coming from a sister genus of *Oregano*, but is now officially a species of oregano itself[1]. In New Zealand the names are often used interchangeably, though *marjoram* (also known as sweet marjoram) differs from oregano in having a milder flavour. *Oregano* is one of the most studied herbs, as it has shown consistently high levels of phenolics, antioxidant activity[2-5], and in food systems has been shown to extend shelf life particularly of oils, but also of foodstuffs containing lipids, such as meat patties[6]. Oregano similarly ranked very highly in a number of studies over a range of different antioxidant assays, demonstrating its various modes of antioxidant activity[7]. Later studies have also found that oregano or oregano extracts exhibited the antioxidant properties in processed foods by retarding lipid peroxidation in edible oils[8] and performing in this regard at least as well as, but usually better than the synthetic antioxidants BHA and BHT. Although most research interest has centred upon oregano as an essential oil. The anti-microbial qualities of oregano have also been investigated. The inhibitory effect of the essential oil of this plant tested on some strains, yeast and fungi may due to its high content of terpinene-4-ol[9]. Phenolics as efficient free radical scavengers they can potentially interact with biological systems and play a role in anticarcinogenic, antiatherogenic, anti-inflammatory, antimicrobial and antioxidant activities[10] . Since the prevention of chronic diseases is a more effective strategy than their treatment, reducing the risk of diseases such as cardiovascular disease and cancer is a subject of great interest for doctors, scientists in general, consumers and the food industry[11] For this reason, many functional foods are nowadays.

2. MATERIALS AND METHODS

2.1. Plant and extraction

2.1.1. Vegetable Matter

The two species of *Origanum family* Lamiaceae were cultivated in the area of El Oued, south of Algeria.

2.1.2. Isolation of the Essential Oils

Leaves of *Origanum vulgare* were placed in the distillation flask of Clevenger apparatus, after 2.5 h of distillation, the volatile oil was collected dried over anhydrous sodium sulfate and stored at 4°C.

2.1.3. Ethanolic extract

The powder of each plant material (10 g) was extract with 135 ml of ethanol absolute into the Soxlet apparatus, and were extracted for 3 hours. The liquids extracts were filtered by

Whatman. The filtrate was concentrated under reduced pressure at 40 °C by rotary evaporator (BUCHI R-210, Switzerland) to eliminate the ethanol, and stored in -4°C to give a crude extract yielding 0.8165 g for fresh leaves of *Origanum majorana* and 0.711 g for *Origanum vulgare*, diluted in ethanol and distilled water for next concentrations needed in this work.

2.2. Chemicals and Reagents.

Gallic acid, rutin, DPPH, aluminum chloride (AlCl3), anhydrous sodium sulfate, sodium carbonate 20% (Na_2 CO3), Folin_Ciocalteu (F-C) reagent, ascorbic acid, alpha-tocopherol, 95% ethanol, distilled water, were procured from Sigma–Aldrich Inc (Paris, France)

2.3. Determination of Total Phenolic

The total phenolics content was determined by Folin-Ciocalteu colorimetric method[12]. Briefly, 100 µL of sample (diluted solution) and 500 µL of Folin-Ciocalteu reagent were pipetted into an eppendorf tube. The contents were vortexed for 10 s. 2mL of 20% (w/v) sodium carbonate solution was added to stop the reaction, the reaction mixture was incubated for 30min at room temperature; the absorbance was measured at 760 nm. Gallic acid concentrations ranging from 0 to 0.30 mg/mL were prepared, and the calibration curve was obtained using a linear fit (Y= 3.435X, R^2 = 0.992). The samples were analyzed in duplicate. All results presented are means (+SD) and were analysed in three replications.

2.4. Determination of Total Flavonoids.

Total flavonoids were estimated according to the aluminum chloride method[13]. Briefly, 1 mL of each sample and 1mLwere added of AlCl3 (1:20 w/v),After 10 s of vortexing, and left at room temperature for 30 min the absorbance for each sample was measured at 510 nm. Rutin concentrations ranging from 0 to 0,06mg/mL were prepared, and the standard calibration curve was obtained using a linear fit (Y= 14.493x+0.339, R^2 =0.9986). The samples were analyzed in duplicate. All results presented are means (±SD) and were analyzed in three replications.

2.5. DPPH radical scavenging activity

The DPPH· free radical scavenging activity of all the extracts and essential oils was measured according to the Well-known DPPH· test The radical scavenging activity using free-radical DPPH assay determinate using methods described in scientific literature[14-16]. Briefly, 100 µL sample of various concentration of ethanolic extract of *Origanum majorana* (0.312, 0.104, 0.078 and 0.062 mg/l, R^2 =0.938, Y=5.028x+32.69) , 100 µL sample of various concentration of ethanolic extract of *Origanum vulgare* (1.1, 0.55, 0.36, 0.275 and 0.22 mg/l R^2 =0.999, Y=8.718x+26.91) and 100 µL sample of various concentration of essential oil of *Origanum vulgare* (45.05, 67.57, 90.1, 112.62 and 135.15 mg/l R^2 =0.9997,

Y=26.917x+8.7187) was added 1 ml of a DPPH methanolic solution (4,9 mg DPPH in 50 ml methanol 100%). The mixture was vigorously shaken and left to stand in the dark for 30 min at room temperature. The antioxidant activity was then measured by the decrease in absorption at 517 nm using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan)and corresponds to the extract ability to reduce the radical DPPH* to the yellow-coloured diphenilpicryldrazine. The antiradical activity was expressed as IC_{50} (µl/ml), the antiradical dose required to cause 50% and calculated by the following equation:

DPPH scavenging activity (%) = $(A_o - A_1) / A_o \times 100$ (1)

Where A_o is the absorbance of control at 30 min, A_1 is the absorbance of the sample extract at 30 min. All results presented are means (±SD) and were analyzed in three replications.

3. RESULTS AND DISCUSSION

3.1. Extract yield

The results of extract yield for each species of *Origanum* are mentioned in table 1, which shows the extraction yield (g/10 g dry weight), the *origanum majorana* species gives the highest yield (8.16±0,108 %) while the intermediate value (7.11±0.140 %) was obtained from the *Origanum vulgare* extract. The yields of essential oil was obtained by hydro-distillation were also mentioned in table 1 the greatest value was found for *Origanum vulgare* 1.43%. For E.Vagi et al. [17], the mass yield obtained for methanolic extract of leaves *O. majorana* about 9.1 % and Viuda-Martos M et al. [18] found 6.4 % for methanolic extract of *O.vulgare* Results are expressed as the mean ±standard deviation of three independent experiments. Values with different row are significantly ($P < 0.05$).

Table 1. Extraction yield leaves of plante *Origanum* and essential oil.

Vegetable matter	**Essential oil yield (%)**	**yield (%)[a]**	**Yield (%)[b] (2)**	**yield (%) [18]**
Origanum majorana	0.17 ±1.37	8.16±0,108		
Origanum vulgare	1.43± 1.49		7.11±0.140	6.4

a yield in % of ethanolic extract obtained starting from 10g extracted fresh matter, b yield in % of ethanolic extract obtained starting from 10g extracted dry matter.

3.2. Total phenolics and flavonoids contents in the selected plant

The total phenolics and flavonoid contents of ***Origanum*** species were measured using F-C reagent and aluminum chloride methods, respectively.

These results obtained by the Soxhlet extraction using ethanol absolute solvent are presented in table 2.

Table 2. Total polyphenol and flavanoid of ethanolic leaves extract of genus *Origanum.*

Plant species	Polyphenols (mg GAE/g)	Flavanoids (mg RE/g)
Origanum majorana	266.86 ± 1.37	057.55 ± 0.58
Origanum vlgare	194.78 ± 1.49	036.63 ± 0.18

Data are expressed as means ± standard deviation of triplicate samples. Values with different row are significantly ($P < 0.05$).

As can be seen from the table 2, significant Phenolics content was observed for different ethanolic extract of *Origanum majorana* (266.86 mg GAE/g,) and *Origanum vulgare* 194.78 mg GAE/g) these concentrations significantly higher if are compared to other medicinal plants like *G. multifolial* 12.36 mg GAE/g and *G. villosa* 20.81 mg GAE/g [19], 70.07 mg GAE/g DW for *M. edule* [20]. According to Zheng & Wang [21], two oregano species tested (*Origanum vulgare and Origanum majorana)* both had extremely high levels of phenolics as well as antioxidant activity. According to the results of the ethanol extract of *Origanum* is poor in flavonoids and is rich in polyphenols. The mean values of total flavonoids content varied from 36.63 to 57.55 mg RE/g, the highest flavonoid contents were found in *Origanum majorana* 57.55 mg RE/g the second was ***Origanum*** *vulgare* 36.63 mg RE/g. The amount of flavanoid was highly considered if it was compared to those obtained in recent studies. For example, the total flavanoid content in *Pinellia ternate* (leaf)1.05 ± 2.93 mg RE/g and *Scutellaria baicalensis* 25.46 ± 4.89 mg RE/g [22], about *A.Vulgaris* the value of total flavonoid is 2.07 ± 0.025 [23] . According to the results of the ethanol extract of Origanum is poor in flavonoids and is rich in polyphenols, generally all plants of the lamiaceae family are known for their phenolic compounds this is in accordance with our results. The flavanoid components have a remarkable activity against several Gram-positive bacteria, such as *Staphylococcus aueus* and Gram-negative, such as *Escherchia coli* [24].

3.4. Free radical DPPH scavenging assay

The DPPH radical scavenging activity of ethanolic extract leaves of the two species of *Origanum* and the DPPH radical scavenging activity of essential oil of *O. Vulgare* are presented in Table 3. For ethanolic extract of *O. majorana* obtained the higher value (IC_{50}=1.37 ± 0.08 mg/L), the intermediate value found in *Origanum vulgare* (IC_{50}=1.53 ± 0.07 mg/L) and the lowest amount obtained from essential oil (IC_{50}=15.360 ± 0.30 mg/L). The antioxidant capacity of the two species of *Origanum* is higher than the positive control BHA (IC_{50} = 28.27 ± 3.85mg/L), this antioxidant capacity free radical scavenger DPPH related with the quantity of total polyphenol composition [25]. The relationship is related to their ability to antioxidant activity, free radical scavenger [26]. Similar results were observed in relation to lard [27, 28].

Though this was not observed by Kulisic et al [29], who showed that various essential oils of various herbs, including oregano, performed less well than ascorbic acid and alpha-tocopherol. The IC50 values are inversely proportional to the anti-radical activity. The values of all ARP (power anti radical activity, ARP=1/ IC_{50} [30].) extracts are significant, moreover, these values do not tent and away from zero. The more ARP increases we can say that our extracts have antioxidant activity. All IC_{50} Are very low ranging between 13.7 and 28.27 µg/ml, under this setting sequestration capacity radical are listed in order:

Origanum majorana > *Origanum vulgare* > essential oil > α tocopherol > BHA

Table 3. DPPH radical scavenging activity (IC_{50} in µg/ml) of the three extracts, ARP and authentic standards

Extracts and standards	DPPH test (IC_{50} in µg/ml)	ARP*
Origanum majorana	13.7 ± 0.08	0.729
Origanum vulgare	15.3 ± 0.07	0.653
BHA	28.27 ± 3.85	0.035
α tocopherol	15.99 ± 0.25	0.062
Essential oil	15.36 ± 0.30	0.0651

Data are expressed as means ± standard deviation of triplicate samples. Values with different row are significantly ($P < 0.05$).* anti-radical activity

4. CONCLUSION

We think that the present study is the first to investigate the antioxidant activity of extracts of *Origanum* genus grown in Southeast of Algeria. The results obtained showed that the extracts ethanolic of *Origanum majorana, Origanum vulgare* and essential oil posses antioxidant activity when compared to standards antioxidant compounds such as BHA and alpha-tocopherol . The values of all ARP (ARP=1/ IC_{50}) extracts are significant. The value ARP of essential oil extract (*Origanum vulgare*) is the smallest. It can be concluded that ethanolic extracts of *Origanum* genus can be used as an accessible source of natural antioxidants with consequent benefits.

5. REFERENCES

[1] McGee H. On food and cooking. Revised ed. New York, Scribner, 2004, 884p.

[2] Capecka E, Mareczek A, Leja M. Antioxidant activity of fresh and dry herbs of some Lamiaceae species. Food Chemistry, 93(2), 2005, 223-226.

[3] Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several Culinary and Medicinal Herbs Are Important Sources of Dietary Antioxidants. Journal of Nutrition 133(5), 2003, 1286-1290.

[4] Shan B, Cai YZ, Sun M, Corke H. Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. Journal of Agricultural and Food Chemistry 53(20), 2005, 7749-7759.

[5] Yanishlieva NV, Marinova E, Pokorný J. Natural antioxidants fromherbs and spices. European Journal of Lipid Science and Technology, 108(9), 2006, 776-793.

[6] Vichi S, Zitterl-Eglseer K, M. J, Franz C. Determination of the presence of antioxidants deriving from sage and oregano extracts added to animal fat by means of assessment of the radical scavenging capacity by photochemiluminescence analysis. Nahrung/Food, 45(2), 2001, 101-104.

[7] Pellegrini N, Serafini M, Salvatore S, Del Rio D, Bianchi M, Brighenti F .Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different in vitro assays.Molecular Nutrition & Food Research, 50(11), 2006, 1030-1038.

[8] Beddows CG, Jagait C, Kelly MJ. Preservation of alpha-tocopherol in sunflower oil by herbs and spices. International Journal of Food Sciences and Nutrition, 51(5), 2000, 327-339.

[9] Benchikha N., these doctorat es –sciences,Univ. Biskra, 2010.

[10] Akanitapichat P., Phraibung K., Nuchklang K., Prompitakkul S., Antioxidant and hepatoprotective activities of five eggplant varieties; Food and Chemical Toxicology, 48, 2010,3017–3021 .

[11] Mandel S, Amit T, Reznichenko L, Weinreb O, Youdim MBH. Green tea catechins as brain-permeable, natural iron chelators-antioxidants for the treatment of neurodegenerative disorders. Molecular Nutrition & Food Research, 50(2), 2006, 229-234.

[12] Moreira L, Dias L G, Pereira J A et al. Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. Food. Chem Toxicol 46, 2008, 3482-3485.

[13] Liu H Y, Qiu N X, DingH H et al. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Res Int 41, 2008, 363–370.

[14] Hatano T, Kagaw H, Yasuhara T et al. Two new flavanoid and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm Bull, 36, 1989 2090-2097.

[15] Falleh F, Ksouri K, Oueslati S et al. Interspecific variability of antioxidant activities and phenolic composition in Mesembryanthemum genus. Food. Chem Toxicol 47, 2009, 2308-2313.

[16] Laouin S. E., Segni L., Gherraf N., Mokni S., J. Fund. App. Sci., 2012, 4(2).48-58.

[17] Vagi, Rapavi, M. Hadolin, K. Vâsârhelyiné Perédi, A. Balâzs, A. Blâzovics ET B. Simândi. Phenolic and Triterpenoid Antioxidants from Origanum majorana L. Herb and Extracts Obtained with Different Solvents. J. Agric. Food Chem., 2005, 53 (1), pp 17-21

[18] Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Perez-Alvarez JA. Antifungal activities of thyme, clove and oregano essential oils. Journal of Food Safety, 27(1), 2007, 91-101.

[19] Liu H Y, Qiu N X, DingH H et al. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Res Int 41: 363–370, 2008.

[20] Falleh F, Ksouri K, Oueslati S et al. Interspecific variability of antioxidant activities and phenolic composition in Mesembryanthemum genus. Food. Chem Toxicol 47: 2308-2313, 2009.

[21] Zheng W, Wang SY 2001. Antioxidant Activity and Phenolic Compounds in Selected Herbs. Journal of Agricultural and Food Chemistry 49(11), 2001, 5165-5170.

[22] Lin Zhang, Ravipati A.S., Antioxydant and Anti-inflamatory Acttivities of Sselected Medicinal Plants Containing Phonolic and Flavonoid Compounds. J. Agric. Food Chem., 2011, 59, 12361-12367.

[23] Ivana Karabegovic, M.Niikolova and al, Chinese Journal of Chemical Engineering, 2011, 19(30), 504-511

[24] Maria Daglia. Polyphenols as antimicrobial agents. Curr Opin. Biotech. 23: 174-181, 2011.

[25] Julia V, Mario R, Maria Cecili L, Polyphenol input to the antioxidant activity of yerba mate (Ilex paraguariensis) extracts. LWT-Food Sci. Technol, 2012:45, 28-35.

[26] Neha B, Harinder S O, Dewinder S U et al. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. Food Res. Int, 2011:44,391-396.

[27] Vichi S, Zitterl-Eglseer K, M. J, Franz C. Determination of the presence of antioxidants deriving from sage and oregano extracts added to animal fat by means of assessment of the radical scavenging capacity by photochemiluminescence analysis. Nahrung/Food, 45(2), 2001, 101-104.

[28] Paula C. Castilho*, Sonia Savluchinske-Feio, Tatiana S. Weinhold, Sandra C. Gouveia P.C. Castilho et al. Evaluation of the antimicrobial and antioxidant activities of essential oils, extracts and their main components from oregano from Madeira Island, Portugal, Food Control 23, 2012, 552-558

[29] Kulisic T, Radonic A, Milos M 2005. Inhibition of lard oxidation by fractions of different essential oils. Grasas Y Aceites, 56(4): 284-291.

[30] Markowicz Bastos, D. H., Saldanha, L. A., Catharino, R. R., Sawaya, A.C.H. F., Cunha, I B. S., Carvalho, P. O. Eberlin, M. N., Phenolic Antioxidants Identified by ESI-MS from Yerba Maté (*Ilex paraguariensis*) and Green Tea (*Camelia sinensis*) Extracts. *Molecules.* 12, 2007, 423-432.

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