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Original Article

IN VITRO EVALUATION OF ANTIOXIDANT CAPACITY OF ALGERIAN ORIGANUM PLANT BY SPECTROPHOTOMETRICAL AND ELECTROCHEMICAL ASSAYS

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ABSTRACT

Objective: The aim of the present study was to evaluate the antioxidant capacity and total phenolic of ethanolic extracts of two plants; *Origanummajorana* and *Origanumvulgare*.

Methods: Total phenolic was estimated by the Folin-Ciocalteu method. The antioxidant capacity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method for spectrophotometrical technique and cyclic voltammetry for electrochemical assay.

Results: 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 mg/L and IC_{50}= 1.53 mg/L) for species *majorana,* and *vulgare* respectively. The antioxidant capacity of two species of *Origanum* was measured using ascorbic acid equivalent antioxidant capacity assays. The *O. majorana* extract showed the highest antioxidant capacity (292.97mg/g) followed by *O. vulgare* extract (163.64mg/g).

Conclusion: The results show that the antioxidant capacity expressed in terms of ascorbic acid equivalent antioxidant capacity obtained from electrochemical experiments (cyclic voltammetry) is higher than that obtained from spectrophotometrical experiments using DPPH. This outcome can be attributed to the overestimation of the total polyphenolic contents due to the interferences of other non-phenolic species.

Keywords: *Origanummajorana, Origanumvulgare*, DPPH, Voltammetry cyclic.

INTRODUCTION

Marjoram was formerly classified as coming from a sister genus of *Oregano*, but is now officially a species of *Oregano* itself [1]. In New Zea land 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 both had extremely high levels of phenolics as well as antioxidant activity. *Oregano* similarly ranked very highly in a number of studies over a range of different antioxidant assays, demonstrating its various modes of antioxidant activity [2-4]. A range of phenolic compounds has been identified in *Oregano* including *rosmarinic*, *caffeic*, *p-coumaric* acids and *caffeoyl* derivatives, the phenolic monterpenes, *carvacrol*, *thymol*, flavonoids, luteolin, apigeninmyricetin and quercetin [5-10]. The aim of this work is to measure the *IN VITRO* antioxidant capacity of the ethanolic extracts of south Algerian Origanum. We used the following two assays systems (i) DPPH radical scavenging activity assay and(ii) cyclic voltammetry assay[11-14]. Total phenolic contents of ethanolic extract of *Origanum majorana* and *Origanum vulgre* were determined by standard colorimetric method.

MATERIALS AND METHODS

Vegetable matter

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

Instrument: UV-Visible spectrophotometer

(PRIM Advanced SCHOTT Instruments Gmbh), centrifuge machine (SLW centryge Ultra-8TL), PGP301 potentiostat with voltamaster 4 version 7.08 software (radiometer analytical SAS), rotary evaporator (IKA evaporator RV 06-ML).

Ethanolic extracts

The powders of each plant material (10 g) was extract with 135 mL of ethanol absolute into the Soxlet apparatus, and was 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 yielding0.8165 g for fresh leaves of *Origanummajorana*s and 0.711 g for *Origanumvulgare*, diluted in ethanol and distilled water for next concentrations needed in this work.

Chemicals and reagents

Gallic acid, DPPH, anhydrous sodium sulfate sodium carbonate 20% (Na_2CO_3), Folin-Ciocalteu (F-C) reagent, ascorbic acid, ethanol (95%), were procured from Sigma–Aldrich Inc (Paris, France), all other reagents used were of analytical grade.

Determination of total phenolic

The total phenolic contents were determined by Folin-Ciocalteu colorimetric method [15]. 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 seconds. 2 mL of 20% (w/v) sodium carbonate solution was added to stop the reaction. The reaction mixture was incubated for 30 minutes at room temperature; the absorbance was measured at 760 nm. Gallic acid concentrations ranging from 0 to 30mg/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 analyzed in three replications.

Evaluation of antioxidant capacity by spectrophotometrical techniques

Using the free radical scavenging determination

The DPPH free radical scavenging activity of all extracts was measured according to the well-known DPPH test. The radical scavenging activity using free-radical DPPH assay determinate with the method described by Hatano et al [16] and Falleh et al [17]. Briefly, 100 µL sample of various concentration of ethanolic extract of *Origanummajorana* (0.312, 0.104, 0.078 and 0.062 mg/mL, R^2 =0.938, Y=5.028x+32.69) and 100 µL sample of various

concentration of ethanolic extract of *Origanumvulgare* (1.1, 0.55, 0.36, 0.275 and 0.22 mg/mL, R^2=0.999, Y=8.718x+26.91) was added to 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 minutes at room temperature. The antioxidant activity was then measured by the decrease in absorption at 517 nm using UV-Visible spectrophotometer and corresponds to the extract ability to reduce the radical DPPH*to the yellow-coloured diphenilpicryldrazine. The antiradical activity was expressed as IC_{50} (µg/mL), the antiradical dose required to cause 50% and calculated by the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100 (1)$$

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

Evaluation of antioxidant capacity by electrochemical techniques

The measurement of the antioxidant capacity of the studied samples of *Origanum* was performed using an electrochemical method based on cyclic voltammetry techniques. Cyclic voltammetry measurements were performed in an electrochemical cell with a volumetric capacity of 50 mL containing a glassy carbon electrode (GCE) working electrode (radio meter analytical SAS), a Pt wire counter electrode and an Hg/Hg2Cl2 reference electrode (saturated with KCl). The potential was swept in direct scanning mode starting from-200 to +1000 mV with a scanning rate of 100 mV/s. To avoid reducing the sensitivity of the working electrode, the latter was polished after each cycle by rubbing its surface using alumina oxide (particle size 0.3µm) before every electrochemical assay. After polishing it was rinsed thoroughly with bi distilled water for 30 seconds. The antioxidant capacity of the studied samples of *Origanum* was obtained using the current density of the anodic curve of the voltammogram. The calibration graph is obtained by plotting the current density of the anodic curve of the voltammogram of each sample of the standard versus its concentration. Ascorbic acid was used as a standard in the calculation of antioxidant capacity of the studied sample of *Origanum* because of its wide spreading in nature and also because its anodic current density displays excellent linearity toward ascorbic acid concentrations [18-19].

RESULTS AND DISCUSSION

Total phenolic contents in the selected plant

The total phenolic contents of *Origanum* species were measured using F-Creagent method.

These results obtained by Soxhlet extraction using ethanol absolute solvent are presented in table 1.

Table 1: Total polyphenol of ethanolic leaves extracts of genus *Origanum.*

Plant species	Polyphenols (mg GAE/g)
Organum majorana	266.86 ± 1.37
Origanum vulgare	194.78 ± 1.49

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 1, significant phenolic contents were observed for different ethanolic extracts of *Origanummajorana* (266.86 mg GAE/g,) and *Origanumvulgare*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 [20], 70.07 mg GAE/g DW for *M. edule* [17]. According to Zheng & Wang [21], two *Oregano* species tested (*Origanumvulgare and Origanummajorana)* both had extremely high levels of phenolic as well as antioxidant activity.

Free radical DPPH scavenging assay

The DPPH radical scavenging activity of ethanolic extract leaves of the two species of *Origanum*are presented in table2. For ethanolic extract of *O. majorana* obtained the higher value (IC_{50}=1.37 ± 0.08 mg/L), the value found in *Origanumvulgare* (IC_{50}=1.53 ±0.07 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 [22]. The relationship is related to their ability to antioxidant activity, free radical scavenger [23]. Similar results were observed in relation to lard [24-25]. The IC_{50} values are inversely proportional to the anti-radical activity. The values of all ARP (Anti radical activity, ARP=1/IC_{50}) [26] 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 1.37µg/mL and 28.7µg/mL, under this setting sequestration, capacity radical are listed in order:

Origanummajorana>*Origanumvulgare*>αtocopherol> BHA

Table 2: DPPH radical scavenging activity (IC_{50} in µg/mL) of the two extracts and ARP authentic standards

Extracts and standards	DPPH test (IC_{50} in µg/mL)	ARP*
Origanummajorana	1.37 ± 0.08	0.072
Origanumvulgare	1.53 ± 0.07	0.065
BHA[27]	28.27 ± 3.85	0.035
Atocopherol[27]	15.99 ± 0.25	0.062

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

Cyclic voltammetry assay

In order to express the antioxidant capacity of different species of the *Origanum* extracts in equivalent terms of ascorbic acid equivalent antioxidant capacity, different concentrations of the standards ascorbic acid (0.049 to 0.868 g/L) were plotted verses the anodic current density obtained from different cyclic voltammograms at pH 7 in 0.2 M phosphate buffer solution as a supporting electrolyte using a 3 mm-diameter glassy carbon electrode present typical irreversible oxidation processes with the existence of an irreversible one oxidation peak at 260mV (figure 1). As can be seen there is an increase in peak current with the increase in ascorbic acid concentrations which leads to a linear relation between these two parameters [28].

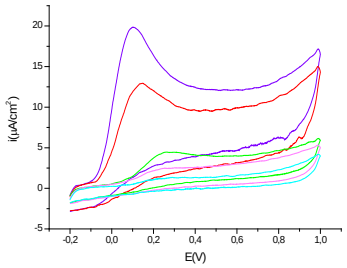

Fig. 1: Cyclic voltammogram referring to different ascorbic acid concentrations

The equation obtained from the linear calibration graph in the studied concentration range for ascorbic acid is, y = 127.39x + 0.12 (where y represents the value of the anodic current density and x, the value of standards concentration, expressed as g/L), with a correlation coefficient ofR^2 = 0.998. The total antioxidant capacity was calculated based on the following equation:

$$TAC = \frac{C_1}{C_2} (2)$$

Where TAC is total antioxidant capacity, C_1 is the *Origanum* sample extract concentration (g/mL), C_2 is the sample concentration in the electrochemical cell (g/mL) calculated by replacing the current density obtained from different voltammograms of sample extracts in the equation y = 127.39x + 0.12. Antioxidant capacity of two *Origanum* varieties from [5, 7] showed higher antioxidant capacity.

Evaluation of antioxidant capacity of *Origanummajorana*

As it can be seen from voltammogram of figure 2, the ethanolic extract of *Origanummajorana* do not response in the same manner as the standard ascorbic acid. The voltammogram of extract represent two peaks for oxidation at 239.9 mV for first oxidation and another for second oxidation at 674.01 mV. This irreversible electrochemical behavior of ethanolic extract may indicate that the *O. majorana* extract contain a different polyphenolic contents of that of the standard ascorbic acid. Electrochemical data calculated from voltammetric measurements of voltammogram of figure 2 are presented in table 3.

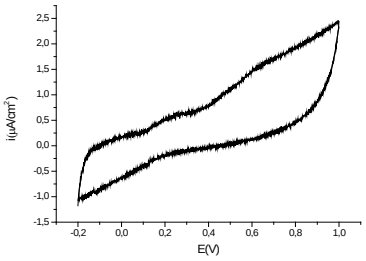

Fig. 2: Cyclic voltammogram of *Origanummajorana* ethanolic extract in pH 7, 0.2M phosphate buffer solution at scan rate 100 mV/s.

Table 3: Electrochemical data of ethanolic extract extracts of *O. majorana*

Ethanolic extract	Epa (mV)*	ipa* (µA/cm²)	Epa** (mV)	ipa** (µA/ cm²)
O. majorana	239.9	0.5504	674.01	1.4406

*First oxidation **second oxidation

Evaluation of antioxidant capacity of *Origanumvulgare*

Figure 3 indicate that ethanolic extract of *Origanumvulgare* present the same irreversible electrochemical behavior of that of the standard ascorbic acid, although with oxidation potential value is less positive than ascorbic acid, around 13,4 to 212 mV. This may indicate that, under this electrochemical conditions, the extract of *O. vulgre* contain the same polyphenolic contents of the standards ascorbic acid. Electrochemical data obtained from voltammogram of ethanolic extract of *O. vulgare* sample is summarized in table 4.

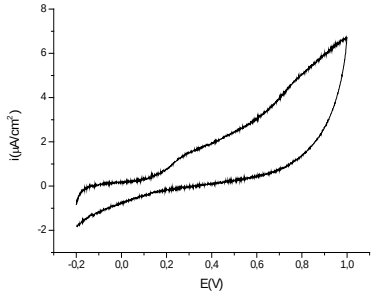

Fig. 3: Cyclic voltammogram of *Origanumvulgare* ethanolic extract in pH 7, 0.2M phosphate buffer solution at scan rate 100 mV/s.

Table 4: Electrochemical data of ethanolic extract of *O. vulgare*

Ethanolic extract	Epa (mV)	ipa* (µA/cm²)
O. vulgare	212	0.8613

The ascorbic acid equivalent antioxidant capacity of different species of the two studied samples extracts calculated from the calibration graphs is summarized in table 5.

Table 5: Antioxidant capacities of two different varieties of *Origanum*

variety	Ascorbic acid antioxidant capacities
	Ethanolic extracts (mg/g)
O. majorana	262.97
O. vulgare	163.64

The ascorbic acid equivalent antioxidant capacity values for different variety of ethanolic extract of studied *Origanum* indicate that *Origanummajorana* variety was the most effective with the highest ascorbic acid equivalent antioxidant capacity value (262.97 mg/g), followed by *Origanumvulgare* variety, ascorbic acid equivalent antioxidant capacity value (163.64mg/g).

CONCLUSION

Both the spectrophotometrical (DPPH) and electrochemical (ascorbic acid equivalent antioxidant capacity) assays suggest that ethanolic extract of two species of *Origanum* shows *IN VITRO* antioxidant activities by inhibiting DPPH which may be due to presence phenolic compounds found in preliminary phytochemical screening. The results also show that the antioxidant capacity of *Origanummajorana* expressed in terms of ascorbic equivalent antioxidant capacity (AEAC) is higher than that obtained for *Origanumvulgare*. Also the results show that the antioxidant capacity expressed in terms of ascorbic acid equivalent antioxidant capacity obtained from electrochemical experiments (cyclic voltammetry) is higher than that obtained from spectrophotometrical experiments using DPPH. This outcome can be attributed to the overestimation of the total polyphenolic contents due to the interferences of other non-phenolic species.

CONFLICT OF INTERESTS

Declared None

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