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Antioxidant activity and quantification of phenolic compounds in methanolic extract of *Origanum majorana* L.

Chaima Farhi [1,*], Ouided Saïdi [1], Amina Gherairia [2], Leila Derradji [1] and Youcef Hadef [2]

[1] *Pharmacognosy Laboratory, Department of Pharmacy, Faculty of Medicine, Badji Mokhtar University, Annaba, Algeria.*
[2] *Laboratory of Analytical Chemistry, Department of Pharmacy, Faculty of Medicine, Badji Mokhtar University, Annaba, Algeria.*

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Abstract

Origanum majorana L. is an aromatic species extensively utilized in traditional medicine due to its broad spectrum of biological activities. The present study was conducted to assess the antioxidant potential of the methanolic extract derived from the aerial parts of this plant. Total polyphenol content was determined using the Folin–Ciocalteu method, while total flavonoid content was quantified via the aluminum chloride colorimetric assay. Antioxidant capacity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. The results revealed that the methanolic extract contains a high level of phenolic compounds, with a concentration of 210.89 ± 3.36 mg GAE/g DW, and flavonoids, with a concentration of 68.61 ± 2.40 mg QE/g DW. The DPPH assay demonstrated notable antiradical activity of the extract, with an IC_{50} value of 183.7 µg/mL, compared with ascorbic acid used as a reference standard, which exhibited an IC_{50} value of 5.68 µg/mL. These findings highlight the antioxidant potential of *Origanum majorana* L. and indicate that this activity is closely associated with its high phenolic content. Consequently, this plant may constitute a promising natural source of bioactive compounds with potential pharmacological applications.

Keywords: *Origanum majorana* L.; Antioxidant Activity; DPPH; Total Polyphenols; Flavonoids; Methanolic Extract.

1. Introduction

The genus *Origanum* comprises 43 species and 18 hybrids distributed across three groups and ten sections. This genus encompasses a wide range of species, subspecies, varieties, and hybrids that can be individually distinguished, although considerable taxonomic variability persists. The genus *Origanum* is broadly distributed from the Canary Islands and the Azores across Northern Europe and extending to East Asia. It is also cultivated in regions such as Cuba and Réunion Island, whereas the Mediterranean basin represents its primary center of distribution [1,2]. Among the species belonging to this genus, *Origanum majorana* L., commonly known as sweet marjoram, is a perennial aromatic plant whose natural distribution is mainly concentrated in North Africa, particularly in Algeria and Tunisia. It typically grows in dry, rocky, and calcareous habitats at altitudes ranging from 100 to 1500 meters [3]. From an ethnobotanical standpoint, this species has been used since antiquity in traditional medicine and is recognized for its therapeutic applications in the treatment of digestive and respiratory disorders, microbial infections, and various dermatological conditions [4,5].

From a phytochemical perspective, *O. majorana* is primarily valued for its essential oil, although additional bioactive secondary metabolites have also been reported [6]. These compounds are mainly concentrated in the aerial parts of the plant, including flowers, stems, leaves, and seeds. The principal constituents of the essential oil of *O. majorana* include terpinen-4-ol (31.15%), cis-sabinene hydrate (15.76%), p-cymene (6.83%), sabinene (6.91%), trans-sabinene hydrate (3.86%), and α-terpineol (3.71%) [7]. Furthermore, polyphenolic secondary metabolites have been identified, including

* Corresponding author: Chaima Farhi

flavonoids and phenolic acids, particularly rosmarinic acid, which is widely recognized for its pronounced antioxidant properties [8]. Within this context, the present study aims to determine the total phenolic and flavonoid contents of the aerial parts of *Origanum majorana* L., as well as to evaluate their antioxidant activity. The antioxidant potential was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, a widely applied method for evaluating the capacity of plant extracts to neutralize free radicals.

2. Materials and methods

2.1. Plant material

The plant material consisted of the aerial parts (leaves and flowering tops) of sweet marjoram *Origanum majorana* L., collected in June 2024 during the flowering period from a natural site located far from any potential sources of pollution. The plant was harvested in the commune of Ouled Driss, Souk-Ahras province. After collection, the aerial parts were subjected to shade drying in a dry, well-ventilated environment for a period of fifteen days. The dried material was subsequently ground into a fine powder and stored under dry conditions, protected from humidity.

2.2. Preparation of the extract

The methanolic extract of *Origanum majorana* L. (Lamiaceae) was prepared following the method described by Motamed [9]. Twenty grams of powdered plant material were extracted with 200 ml of methanol, corresponding to a plant material/solvent ratio of 1/10 (w/v). Extraction was carried out by maceration under continuous agitation at room temperature for 24 hours. The resulting mixture was subsequently filtered through Whatman No. 1 filter paper. The obtained filtrate was concentrated by solvent evaporation using a rotary evaporator at 40 °C until a dry extract exhibiting a green coloration and oily consistency was obtained.

2.3. Determination of total polyphenols

The total polyphenol content was determined spectrophotometrically using the Folin–Ciocalteu colorimetric method [10]. For each dilution of the methanolic extract, 200 µl were transferred into a test tube and supplemented with 1 ml of Folin–Ciocalteu reagent previously diluted tenfold. After 5 minutes, 800 µl of a 7.5% sodium carbonate (Na_2CO_3) solution were added to the reaction mixture. Following homogenization, the tubes were incubated at room temperature in the dark for 30 minutes. Absorbance was then measured at 765 nm against a blank using a spectrophotometer. In parallel, under identical experimental conditions, a calibration curve was constructed using various concentrations of gallic acid as a reference standard. The total phenolic content was calculated and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g dry extract) [11].

2.4. Determination of flavonoids

The flavonoid content was determined using a spectrophotometric method based on the formation of a stable yellow complex resulting from the interaction between aluminum chloride and the oxygen atoms at positions C-4 and C-5 of the flavonoid structure [12]. For each dilution of the previously solubilized crude methanolic extract, 1 ml was transferred into a test tube, followed by the addition of 0.5 ml of 2% $AlCl_3$ solution and 0.5 ml of potassium acetate solution. The mixture was then incubated for 10 minutes in the dark, after which absorbance was measured at 415 nm against a blank using a spectrophotometer. The blank was prepared under identical conditions, with methanol replacing the extract [13]. In parallel, a calibration curve was established using different concentrations of quercetin as a reference standard. Based on this calibration curve, the flavonoid content was calculated and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g dry extract).

2.5. Evaluation of antioxidant activity: DPPH assay

The method is based on the evaluation of the ability of antioxidants to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH●). This radical is reduced to its corresponding hydrazine (non-radical) form through hydrogen atom donation. The presence of DPPH● in solution imparts a deep purple coloration with strong absorbance at 517 nm. Upon reaction with antioxidant compounds, this coloration progressively fades due to the reduction of the radical form, leading to a decrease in absorbance [14]. For the assay, 1 ml of different concentrations of the extract was mixed with 2 ml of freshly prepared methanolic DPPH solution (0.04 g/L). After incubation in the dark for 1 hour and 30 minutes at room temperature, absorbance was recorded at 517 nm against a blank using a spectrophotometer. The negative control was prepared under identical conditions by mixing 1 ml of methanol with 2 ml of methanolic DPPH solution. Ascorbic acid was used as a positive control [15].

The percentage of inhibition was calculated using the following equation:

$$\mathbf{I\ \%} = ((Ac - At) / Ac) \times 100$$

I%: percentage of inhibition
Ac: absorbance of the control
At: absorbance of the test sample

3. Results

3.1. Polyphenol determination

The calibration curve of gallic acid (Figure 1) exhibited strong linearity (R^2 = 0.9978), enabling reliable quantification of total polyphenol content based on absorbance measurements at 765 nm. The mean total polyphenol content of the methanolic extract of *Origanum majorana* L. was 210.89 ± 3.36 mg GAE/g DW.

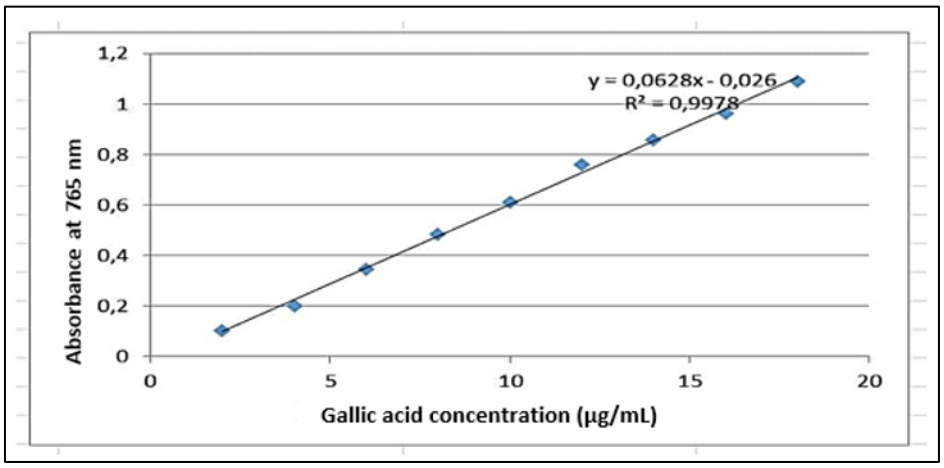

Figure 1 Calibration curve of gallic acid for the determination of total polyphenols

3.2. Flavonoid determination

The calibration curve of quercetin (Figure 2) demonstrated a linear detector response across the tested concentration range, with a correlation coefficient of R^2 = 0.999. The mean flavonoid content was 68.61 ± 2.40 mg QE/g DW.

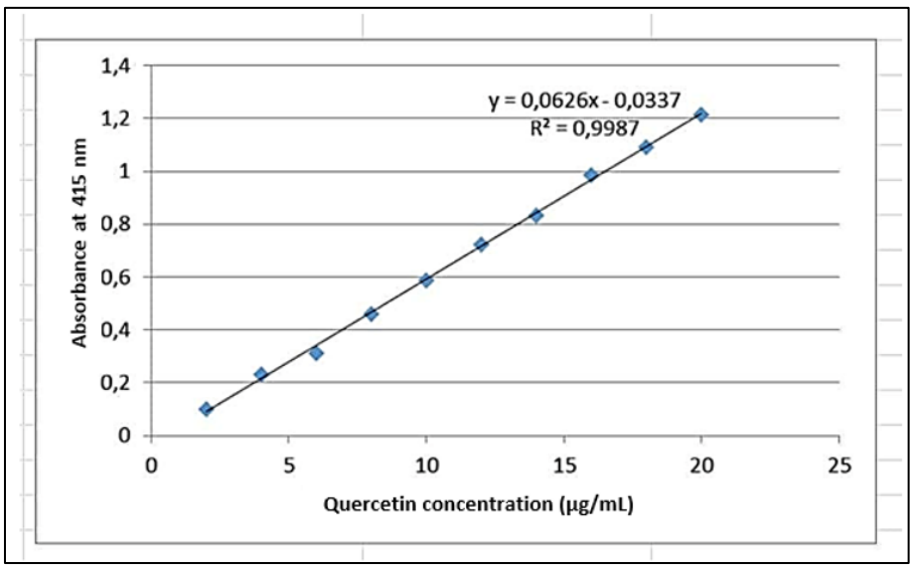

Figure 2 Calibration curve of quercetin for the determination of flavonoids.

3.3. Antioxidant activity

A progressive color transition from purple to yellow was observed in the DPPH solution in the presence of increasing concentrations of the extract (Figure 3), indicating a positive reaction between DPPH and the methanolic extract of *Origanum majorana* L. This color change reflects the reduction of DPPH● to 2,2-diphenyl-1-picrylhydrazine (DPPH-H) by hydrogen-donating antioxidant compounds present in the extract, resulting in a decrease in absorbance at 517 nm.

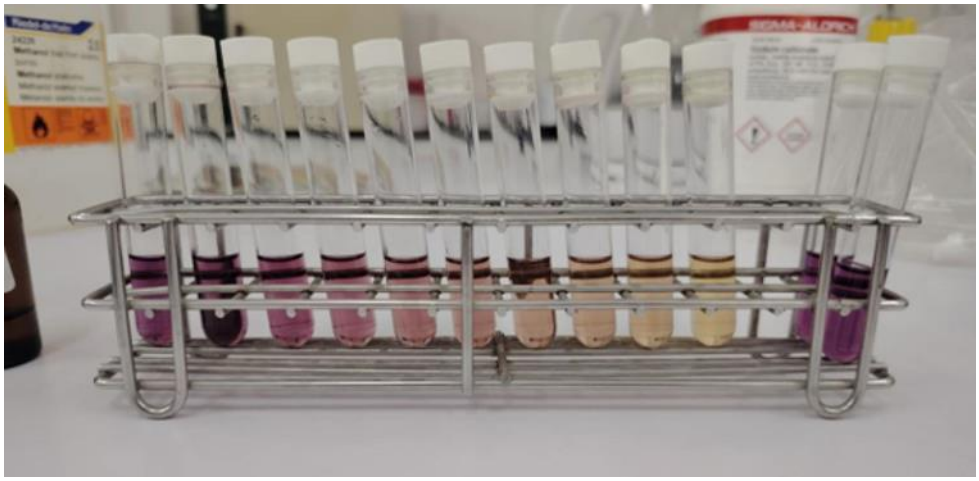

Figure 3 Reaction of the DPPH solution with different dilutions of the methanolic extract of *Origanum majorana* L**.**

From the inhibition curves of DPPH radical scavenging activity as a function of extract and ascorbic acid concentrations in methanol:

- The IC_{50} value of ascorbic acid (reference synthetic antioxidant) was determined as IC_{50} = 5.68 µg/ml (Figure 4).

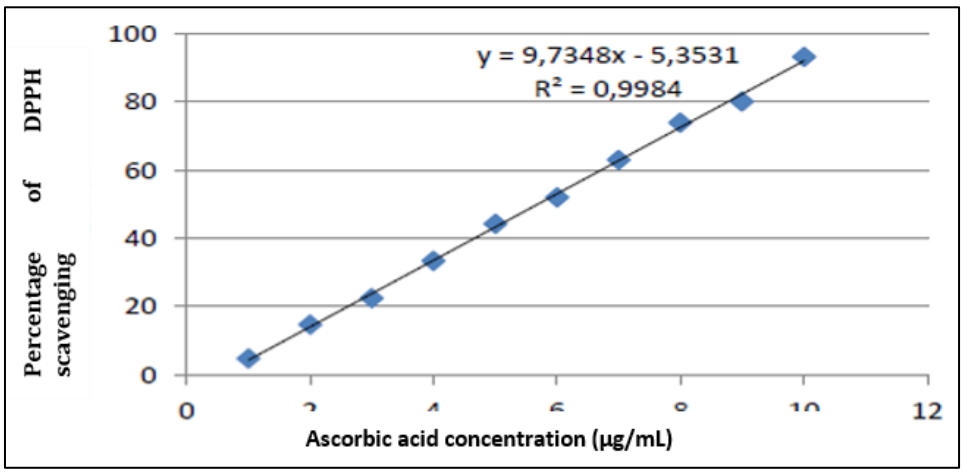

Figure 4 Evaluation of the percentage inhibition of the DPPH radical as a function of ascorbic acid concentration (correlation coefficient = 0.9984).

- The IC_{50} value of the methanolic extract of *Origanum majorana* L. was determined to be 183.7 µg/ml (Figure 5).

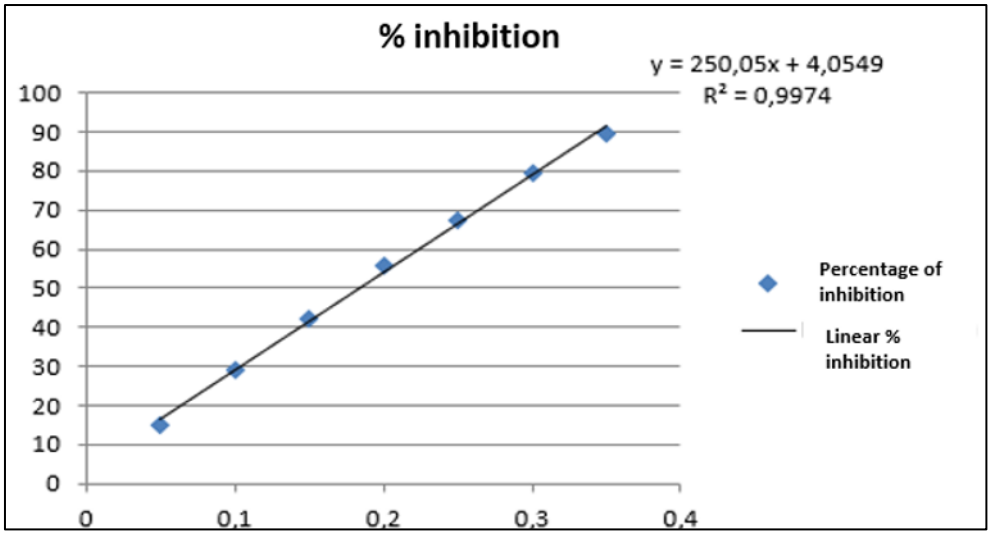

Figure 5 Evaluation of the percentage inhibition of the DPPH radical as a function of the concentration of the methanolic extract of marjoram (correlation coefficient = 0.9974).

Based on the results presented in Figure 4, the antioxidant activity appears to be concentration-dependent, increasing proportionally with rising extract concentrations. The methanolic extract exhibited moderate antioxidant activity against the DPPH radical, with an IC_{50} value of 183.7 µg/ml. However, this activity remains considerably lower when compared with the reference synthetic antioxidant, vitamin C (IC_{50} = 5.68 µg/ml).

4. Discussion

The total phenolic content obtained in the present study (210.89 ± 3.36 mg GAE/g DW) is slightly lower than that reported by Bouyahya et al. (266.86 mg GAE/g DW), while remaining higher than values reported by El Amiri MA for an aqueous extract (186.06 ± 0.1 mg GAE/g DW) and by Lahrech et al. for an ethanolic extract (164.96 mg GAE/g DW) [16,17].

Flavonoid quantification indicated that the aerial parts of *O. majorana* L. contain an average content of 68.61 ± 2.40 mg QE/g DW. This value is comparable to that reported by El Amiri MA (2025), who recorded 72.3 ± 0.9 mg QE/g DW in the aqueous extract of marjoram [17]. The present results are clearly higher than those reported by Lahrech et al. (2020), who found 44.61 ± 2.08 mg QE/g DW [18].

The variations observed between different studies may be attributed to multiple factors, including geographical origin, climatic conditions, developmental stage of the plant, harvesting period, post-harvest storage conditions, and the nature of the extraction solvent. In addition, genetic variability may influence the biosynthesis and accumulation of secondary metabolites. It should also be noted that the Folin–Ciocalteu assay is not entirely specific to phenolic compounds, as it may react with other reducing agents such as ascorbic acid, certain reducing sugars, and additional antioxidant molecules, potentially leading to an overestimation of total phenolic content [19,20].

The IC_{50} value obtained in this study (183.7 µg/ml) is markedly lower than that reported by Ennaji et al. (2308 µg/ml), indicating a comparatively higher antioxidant potential of the present extract [21]. Conversely, studies conducted by Udaya Prakash et al. (2019) and Lahrech et al. (2020) on *Origanum majorana* L. have reported stronger antioxidant activities for ethanolic extracts, with IC_{50} values of approximately 10 µg/ml and 40.09 µg/ml, respectively [18,22].

The relatively high phenolic (210.89 mg GAE/g DW) and flavonoid (68.61 mg QE/g DW) contents determined in the present study may explain, at least in part, the significant antioxidant activity observed.

The antioxidant activity of plant extracts is primarily associated with their richness in phenolic compounds. Among these, flavonoids play a central role due to their ability to neutralize free radicals and reactive oxygen species. This activity is linked to their relatively low redox potential, which facilitates hydrogen atom donation from hydroxyl groups, thereby stabilizing radical species [23]. Accordingly, the observed antioxidant activity may result from the synergistic effect of multiple phenolic constituents present in the extract. In addition, their mechanism of action is not limited to

free radical scavenging. These compounds may also inhibit oxidative enzymes and chelate transition metal ions, thereby reducing the formation of reactive oxygen species.

Overall, these findings suggest that the methanolic extract of *Origanum majorana* L. constitutes a promising natural source of antioxidant compounds, mainly due to its high content of phenolic constituents.

5. Conclusion

The antioxidant potential of the methanolic extract of *Origanum majorana* L. was evaluated through the quantification of phenolic and flavonoid contents, followed by the assessment of antiradical activity using the DPPH assay. The results indicate that the aerial parts of *Origanum majorana* L. exhibit notable antioxidant activity, which is likely associated with their richness in phenolic compounds.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

The present research work does not contain any studies performed on animals/human subjects by any of the authors.

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