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A Multi-Approach Study of Phytochemicals and Their Effects on Oxidative Stress and Enzymatic Activity of Essential Oil and Crude Extracts of *Rosmarinus officinalis*

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Abstract: *Rosmarinus officinalis* or Rosemary is a highly valued medicinal vegetal, owing to its notable antispasmodic, anti-inflammatory, and antibacterial properties. In the current work, we aimed to identify the chemical components of the essential oil (EO) of *R. officinalis* and evaluate its biological properties using an in vitro approach. High performance liquid chromatography time-of-flight mass spectrometry (HPLC-TOF-MS) was utilized to analyze of the hydro-methanolic extract (HME), while gas chromatography–mass spectrometry (GC/MS) was considered during the analysis of the EO's chemical composition. The antioxidant abilities of HME and the EO were assessed using diverse tests (DPPH, ABTS, GOR, CUPRAC, and FRAP). The anti-enzymatic properties were tested by the inhibition of cholinesterases, α-glucosidase, and tyrosinase enzyme. The HPLC-TOF-MS displayed the existence of flavonoids like luteolin glucuronide I and II, and a few known hydroxycinnamic acids. The EO contained three major components, namely, eucalyptol (28.7%), camphor (16.7%), and borneol (13.5%). The HME had a high total polyphenol content, as determined by the Folin–Ciocalteau method (335.37 ± 9.33 µg of gallic acid eq·mg^{-1}). Notably, the analysis of the bioactivities of the HME and EO revealed comparatively that they possessed higher radical scavenging capacity in the DPPH, ABTS, and galvinoxyl assays, while EO exhibited a higher capacity for enzyme inhibition. Overall, our findings suggest that both the EO and HME extract of Algerian's *R. officinalis* holds great usefulness in the pharmaceutical and nutraceutical fields due to its elevated polyphenol content and potent bioactivities.

Keywords: *Rosmarinus officinalis*; polyphenols; essential oil; antioxidant; enzymes inhibition

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1. Introduction

Herbal extracts were commonly utilized in traditional medication for centuries to remedy various dysfunctions or clinical health issues. The cost effectiveness and clinical success of these folk remedies have been well-established, and they generally have fewer side effects when compared to some synthetic drugs. Recently, the pharmaceutical industry has

increased its use of phytochemical components derived from medicinal plants [1]. Plants' secondary metabolites, such as steroids, alkaloids, phenolics, lignans, polysaccharides, and glycosides, have numerous beneficial biological properties [2]. These metabolites are known to act as antimicrobial, anti-inflammatory, anti-allergic, anticancer, antidiabetic, and antioxidant agents. They have also been utilized in the management of diabetes mellitus, a chronic condition that results from metabolic disorders in pancreatic beta cells, leading to hyperglycemia [3]. Additionally, secondary metabolites have shown promise in inhibiting the α-amylase and α-glucosidase involved in carbohydrate digestion, thereby reducing glucose levels and absorption, which affects brain cells and can help manage Alzheimer's disease [4–6]. Prior studies investigated the discovery of natural substitute antioxidants derived from plants and their inhibitory potential on enzymes in relation with Alzheimer's and/or diabetes [7]. The usefulness of medicinal vegetation as spices in culinary practices is also attributed to the properties of their secondary metabolites, which enhance the taste of meats, poultry, and olive oil [8].

Algeria is among the North African countries with the richest vegetation, where herbs are commonly used in folk medicine as part of the population's culture. A recent ethnobotanical survey revealed that the most commonly used plants in Algeria belong to the Asteraceae, Lamiaceae, and Apiaceae families [9]. Rosemary, "*Rosmarinus officinalis* L.", a Lamiaceae taxon, is a perennial Mediterranean herb widely distributed throughout Algeria, where it is known as "Iklil" [10]. This plant is used as a spice in culinary practice and for remedial treatments, such as for digestion and as a carminative for the treatment of colds, and respiratory, gastrointestinal, and liver inflammation. Additionally, it is utilized as an antitumor, antioxidant, anti-inflammatory, and antimicrobial agent [11]. Phenolic extracts are employed as a natural remedy for numerous common diseases [12]. Rosmarinic acid, considered the main rosemary phenolic compound, has many biological properties, including antibacterial, antiviral, antioxidant, and antimutagenic properties [13]. Recently, it was documented that this chemical may be an efficient factor to treat Alzheimer's disease [14]. Results obtained from previous studies on other constituents of this plant, such as carnosic acid and its derivative carnosol, suggest that such components is linked with anti-tumoral and anti-inflammatory properties [15]. Tyrosinase is a metalloprotein in charge of catalyzing the phase of rate-limiting of melanogenesis. When absent or when its functional gene is mutated, pigmentation is reduced or even eliminated. The latter is associated with oculocutaneous albinism (OCA type I) [16]. In the food products sector, tyrosinase is also recognized as the enzyme involved in fruit browning, which occur after the oxidation of phenolic components to quinone [17].

It is hypothesized that the antioxidant activity of Algerian Rosemary HME and EO is related to their main components and these extracts possess further beneficial properties for human health [18]. The present work explored the chemical composition of HME and EO of *R. officinalis* grown in Algeria in order to determine its antioxidant and enzymatic-inhibitory properties.

2. Materials and Methods

2.1. Chemicals

The chemicals used in this work were all analytical grade and the water was Milli-Q (Millipore, Bedford, MA, USA). For LC-MS/MS analysis, formic acid, water, and methanol were Optima™ LC/MS grade (Fisher Scientific, Fair Lawn, NJ, USA). Gradient HPLC grade acetonitrile (MeCN) was obtained from Scharlab (Barcelona, Spain), sodium bicarbonate, Folin–Ciocalteau, triethanolamine (TEA), sodium dodecyl sulfate (SDS), vanillin-tannic acid, acetic acid, urethane AHA, perchloric acid, 2,2-diphenyl-1-picrylhydrazine (DPPH), potassium persulfate, 2,2′-azido-bis-(3-ethylbenzothiazoline-6-diammoniumsulfonic acid (ABTS), disodium hydrogen phosphate, trifluoroacetic acid (TFA), 99%), hydrogen peroxide, ferric chloride, sodium dihydrogen phosphate dihydrate, ferric chloride, trichloroacetic acid (TCA), potassium ferrocyanide (III), ascorbic acid (vitamin C), butylated hydrox-

yanisole (BHA), and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Vegetable Material

The aerial portion of *R. officinalis* was collected in the flowering period (December 2020) from Oum El Bouaghi, Algeria (35°52′31.48″ N, 7°06′48.71″ E; annual rainfall = 25.73 mm; altitude = 902 mm). Botanical determination was confirmed based on available literature and a voucher specimen was submitted to the Herbarium at Constantine University, Algeria (CUTR2020/20). The sampled plant material was first dried for 4 days in the shade in order to maintain intact their bioactive molecules as much as is feasible.

2.3. Hydro-Methanolic Extraction (HME)

The HME was started as reported Mammeri et al. [19], but after minor changes. The powered material (50 g) was macerated with 500 mL of 80% methanol for 1 h at room temperature. The mixture obtained was thereafter filtrated using a 0.45 mm Millipore filter and dried at 40 °C under decreased pressure to acquire a crude extract. The hydromethanolic extract was then preserved in amber tint glass bottles (+4 °C) until ulterior use.

2.4. Essential Oil Extraction (EO)

The hydrodistillation method was carried out using a Clevenger-type apparatus [20], where 50 g of powdered sample was added to 1000 mL flasks filled with 500 mL of distilled water. Next to 3 h of extraction, the EO was recovered in a sealed glass vial and enclosed in aluminum foil to avoid light.

2.5. HPLC-MS-TOF Examination

An Agilent 1200 Liquid Chromatography system (Agilent Technologies, Palo Alto, CA, USA) fitted with a typical autosampler was used to carry out the phenolic characterization. Agilent extended the C_{18} (1.8 µm, 2.1 × 50 mm) HPLC column. Gradient elution was necessary during separation (40 °C) and a flow rate equal to 0.4 mL·min^{-1} was applied. Water and 0.1% formic acid (A) and acetonitrile composed the mobile phases (B).

A multistep linear gradient was considered according to the values: 0 min, 5% B; 10 min, 25% B; 14 min, 50% B; 20 min, 80% B; and 22 min, 90% B. The starting conditions were detained for 5 min. The HPLC system's injection capacity was 5 L. The Agilent 6320 TOF mass spectrometer (Agilent Technologies, Palo Alto, CA, USA), which employed a dual ESI interface and functioned in negative ion mode with a capillary voltage of 3.5 kV, was connected to the HPLC system. The optimum values for the ESI-TOF-MS conditions were set as follows: nebulizing gas pressure 40 psig, drying gas flow 12 L·min^{-1}, and drying gas temperature 300 °C. The detection procedure was started between the mass ranges of 50 and 1700 m·z^{-1}. The precise mass data of the molecular ions have been analyzed using the Mass Hunter software (Agilent Technologies, Palo Alto, CA, USA) according to Nicolì et al. [21].

In this study, gas chromatography–mass spectrometry (GC-MS) was necessary to characterize the essential oil (EO) structure. An Agilent 7890B with a mass-selective detector Agilent5977A was used, along with a bonded silica capillary column Agilent HP-5MS (30 m × 0.25 mm, 0.25 µm film width). Helium was considered as a carrier (flow rate = 2.5 mL·min^{-1}) (Agilent Technologies, Palo Alto, CA, USA). The recognition of EO compounds was conducted by comparing the obtained retention index (RI) and those of reference materials, and mass spectrum data were compared with MS libraries (NIST 14) to confirm the identification. Additionally, gas chromatography with flame ionization detection (GC-FID) was conducted using a PerkinElmer GC-FID system associated to a DB-5 column (30 m × 0.53 mm, film width 1.5 µm) by J&W, USA to assess the EO compounds.

2.6. Antioxidant Activity

Antioxidant activity was determined in *R. officinalis* extracts and EO by means of five different methods [22]: (1) free radical DPPH scavenging, (2) CUPRAC assay, (3) ABTS assay, (4) GOR radical scavenging test, and reducing power (5). The findings were given as IC50 (mg·mL^{-1}) for ABTS and DPPH, A0.50 (µg·mL^{-1}) for the CUPRAC, and FRAP EC50 (µg·mL^{-1}) for the galvinoxyl scavenging assay.

2.6.1. Total Polyphenol Determination

The content of polyphenols was determined using the Folin–Ciocalteau reagent following the microplate assay method reported in Singleton et al. [23]; a total volume of 100 µL of diluted FCR and 20 µL of plant extract (1 mg·mL^{-1}) was placed in the 96-well microplate (1:10), then 75 µL of 7.5% sodium carbonate was added. The mixture was then preserved at room temperature (darkness, 2 h). The absorbance was determined with the aid of a PerkinElmer microplate reader at 765 nm (USA). The linear regression was used to set the calibration curve for gallic acid (y = 0.0034x + 0.1044; R^2 = 0.9972); the result was expressed as µg of gallic acid equivalents per milligram of extract (µg GAE·mg^{-1} E).

2.6.2. DPPH Scavenging Effect

The DPPH free radical scavenging assay was carried out based on the modified protocol outlined by Blois [22]. To prepare the samples, 40 µL of extract (0.4 mM in methanol) was mixed with 160 µL of DPPH solution, and the resulting mixture was put at room temperature (darkness, 30 min). The absorbance was then measured at 517 nm to determine the inhibition capacity of the DPPH radical, with BHT and α-tocopherol considered as reference substances to construct the standard curves. The inhibition capacity was evaluated with the aid of the equation (Ac − At/Ac) × 100, where Ac represents the absorbance of the control reaction and At represents the absorbance of the testing sample. The results were reported as IC50 values, which indicate the concentration of the extracts necessary to achieve a 50% reduction in the absorbance (at 517 nm) in comparison to the control. The standard curves for BHA, BHT, and α-tocopherol were given as follows: for BHA, R^2 = 0.9981, y = 0.7964x + 75.576; for BHT, R^2 = 0.9963, y = 0.2437x + 48.561; and for α-tocopherol, R^2 = 0.9856, y = 0.4943x + 36.342.

2.6.3. ABTS Scavenging Assay

The ABTS radical scavenging activity was carried out as provided by reference [24], with minor changes. Firstly, the ABTS solution has been mixed with potassium persulfate and left for 24 h to obtain ABTS radicals. The mixture was diluted and measured at 734 nm. The samples were left with ABTS buffer for seven minutes and the absorbance was then determined. To calculate the percent inhibition, the formula (Ac − At/Ac) × 100 was used, with Ac being the control absorbance and At being the sample absorbance. BHA, BHT, gallic acid, and quercetin served as the reference standards. The IC50 values were accessed by creating a graph of inhibition versus sample concentration. A linear standard curve between 25 and 600 mM BHA served to obtain the results in millimoles of BHA equivalent (TE) per gram of wet weight. If the ABTS value was higher than the linear reference range, the sample needed to be further diluted [24].

2.6.4. Galvinoxyl Scavenging Assay (GOR)

The antiradical potential of the extracts in contradiction to galvinoxyl radical was evaluated using a modified method described by Shi [25]. Briefly, several concentrations of extract (40 µL) were mixed with galvinoxyl radical solution (160 µL, 10 M) before their incubation at room temperature (2 h). The concentration decline of galvinoxyl radical was evaluated after the measurement of the absorbance at 432 nm using a spectrophotometer. The antiradical activity (EC50) of the extracts was expressed as the concentration of the analyzed extract resulting in a half reduction in the absorbance at 432 nm in comparison to the reference sample. The proportion of galvinoxyl radical scavenging was computed

with the aid of the formula galvinoxyl radical-scavenging (%) = [(A of control − A of the sample)/A of control) 100]. Gallic acid and BHA were considered as reference standards. The percentage of H_2O_2 inhibition was estimated using the formula (Ac − At/Ac) × 100, where At is the absorbance of the sample tested and Ac is the absorbance of the control. The IC50 (the sample concentration that reduces the half of H_2O_2) was evaluated by plotting the obtained inhibitions versus sample concentrations. BHA and BHT were utilized to construct the standard curves (for BHA: R^2 = 0.9962, y = 0.8429x + 24.1133; for BHT: R^2 = 0.9983, y = 0.2594x + 34.1414).

2.6.5. Cupric Ion Reducing Antioxidant Capacity (CUPRAC)

The antiradical activity in opposition to the galvinoxyl radical was measured of the extracts using a modified protocol proposed by Shi [25]. The extracts (40 µL) at various concentrations were mixed with galvinoxyl radical solution (160 µL, 10 M) and incubated at room temperature for 2 h. The decline in galvinoxyl radical amount was determined by spectrophotometry (432 nm). The antiradical activity (EC50) was given as the concentration of the extract that resulted in a half decline in the absorbance at 432 nm in comparison to the reference sample. The proportion of galvinoxyl radical scavenging was determined based on the equation galvinoxyl radical-scavenging (%) = [(A of control − A of the sample)/A of control) 100]. Gallic acid and BHA were considered as standards. The percentage of H_2O_2 inhibition was evaluated using the formula (Ac − At/Ac) × 100, where At is the absorbance of the sample tested and Ac is the absorbance of the control. The IC50 (the sample concentration that reduces the half of H_2O_2 present) was measured after plotting the obtained inhibitions against sample concentrations. The standard curves for BHA and BHT were constructed (for BHA: R^2 = 0.9962, y = 0.8429x + 24.1133; for BHT: R^2 = 0.9983, y = 0.2594x + 34.1414) [25].

2.6.6. Reducing Power Assay (FRAP)

The reducing power assay was performed following the protocol of Oyaizu [25] with minor modifications [26]. The assay involves a combination of several reactions that generate colorful complexes when antioxidants react with potassium ferricyanide, trichloro ferric chloride, and acetic acid. The absorbance of the obtained complexes is determined at 700 nm, and an enhancement in absorbance shows an increase in the reducing potential of the sample. Briefly, 10 µL of the extract was mixed with 50 µL of potassium ferricyanide (1%) and 50 µL of phosphate buffer, and the resulting solution was incubated at 5 °C for 20 min. Then, 50 µL of trichloroacetic acid (10%), 40 µL of distilled water, and 10 µL of ferric chloride were added. The absorbance of the sample was determined at 700 nm, and the results were expressed as ascorbic acid equivalents/mg extract. Quercetin, gallic acid, BHT, and BHA were used as reference standards. The ascorbic acid equivalents were evaluated with the aid of a calibration curve of ascorbic acid (ascorbic acid: y = 0.0078x − 0.0039, R^2 = 0.9986; tannic acid: y = 0.0072x + 0.0291, R^2 = 0.9812 and α-tocophérol: y = 0.0193x − 0.0057, R^2 = 0.9994).

2.7. Anti-Cholinesterase Activity

The anti-cholinesterase activity inhibitions were evaluated using the spectrophotometric method reported in [27], with a few modifications, as described in [28]. Acetylthiocholine iodide and butyrylthiocholine chloride were considered substrates, associated with electric eel and horse serum AChE and BChE, respectively. Cholinesterase activity was evaluated on the basis of DTNB [5,5-dithio-bis (2-nitrobenzoic acid)]. Test substances and controls were dissolved using ethanol as a solvent [29]. The reading was performed using a microplate and the following reagents: Na_2HPO_4, $2H_2O$ NaH_2PO_4, $2H_2O$ $NaHCO_3$, NaOH DTNB ACİ (acetylthiocholine iodide) BuCİ (S-butyrylthiocholine iodide) AChE, BChE, Galantamin, distilled water and ethanol as solvents.

For enzymes, an AChE solution was prepared by combining 0.2 mg of AChE with 4 mL of a pH 8 buffer, resulting in solution A. In a single eppendorf containing 20 µL of

solution A, 3 mL of PH = 8+ was added and the absorbance at 412 nm was to be determined. This value should be in the range of 0.4–0.5. BChE solution: 0.2 mg AChE and 1 mL (pH = 8) were mixed to prepare solution A. A total of 20 µL of solution A was transferred into 20 tubes. Each sample (tube) filled with 20 µL of solution A and 2 mL of pH = 8 was checked in a spectrophotometer at 412 nm, where the absorbance value should fall in the range of 0.4−0.5.

The protocol steps are as follows: A total of 150 µL of sodium phosphate buffer (100 mM, pH 8.0) was mixed with 10 µL of extract solution melt in ethanol at several concentrations and 20 µL of AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U) solution. This combination was incubated at 25 °C (15 min), then 10 µL of DTNB (0.5 mM) and 10 µL of acetylthiocholine iodide (0.71 mM) or 10 µL of butyrylthiocholinechloride (0.2 mM) was added and the absorbance was measured at 412 nm after 0 min, 5 min, 10 min, and 15 min. The percentage of inhibition for BChE and the AChE was evaluated against the blank (i.e., ethanol with phosphate buffer, pH8) and calculated according to the formula $(E - S)/E \times 100$, where E is the activity of the enzyme without extract and S is the enzyme activity with the extract. The concentration needed to reach the inhibition of BChE by 50%, or the IC_{50} was estimated graphically, with galantamine used as a positive control. The hydrolysis of the above substrates was measured spectrophotometrically upon the yellow appearance.

2.8. α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory capacity of the extract was evaluated following the method described by Asghari [30]. The extract solution was incubated with Na_3PO_4 buffer (0.2 M, pH 6.9) at 37 °C (10 min), followed by the addition of p-nitrophenyl-D-glucopyranoside (5 mM) and another incubation at 37 °C for 10 min. The reaction was stopped by adding iodine–potassium iodide solution and 1 M HCl. The percentage of inhibition of α-amylase was calculated by measuring the absorbance at 630 nm and using the formula [(A of control − A of the sample)/A of control]/100. Acarbose was used as a positive control and the IC_{50} value was calculated graphically [30].

[(A of control − A of sample)/A of control]/100 equals α-amylase inhibition%.

Acarbose was considered as a positive control and the IC_{50} concentration that could inhibit α-amylase by 50% was graphically evaluated.

2.9. Tyrosinase Inhibition

The tyrosinase inhibitory impact of the plant extract was evaluated as reported by Deveci [31]. In brief, the reaction mixture containing tyrosinase (2500 $U \cdot mL^{-1}$), phosphate buffer (0.05 M, pH 6.5), plant extract solution (50 µL), and L-DOPA (25 µL, 5 mM) was incubated, and the absorbance was determined at 475 nm to assess dopachrome development. The measurements were started in three replicates, and the concentration–response curves were necessary to deduce the inhibition percentage of tyrosinase activity and the IC50 value. Kojic acid was considered as a positive control in the assay [31].

2.10. Evaluation of Limit of Detection, and Limit of Quantification

LOD (limit of detection) and LOQ (limit of quantification) are considered important parameters in analytical chemistry which indicate the lowest detectable concentration of an analyte that can be quantified by a given method, respectively.

To calculate LOD and LOQ, the following equations are used:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.11. Statistical Analysis

All the parameters considered in triplicate in the current study were given as the mean ± SD. The IC50 (50% inhibition concentration) and A0.5 (the concentration indicating 0.50 absorbance) were estimated with the aid of the linear regressions from the two curves plotted % inhibition = f (concentrations) for IC50 and Absorbance = f (concentrations) for the A0.50. The numerical series obtained were subjected to the analysis of variance (abbreviated 1-ANOVA), followed by multiple comparisons with the aid of Tukey's honestly significant difference (HSD) test. These parametric analyses were carried out using the STATISTICA v8.0 software [32].

3. Results

3.1. HPLC-MS-TOF Analysis

The LC-MS/MS assay was employed to identify the bioactive compounds in *R. officinalis* HME extracts obtained from the areal part of the plant. The identified components are shown in Figure 1.

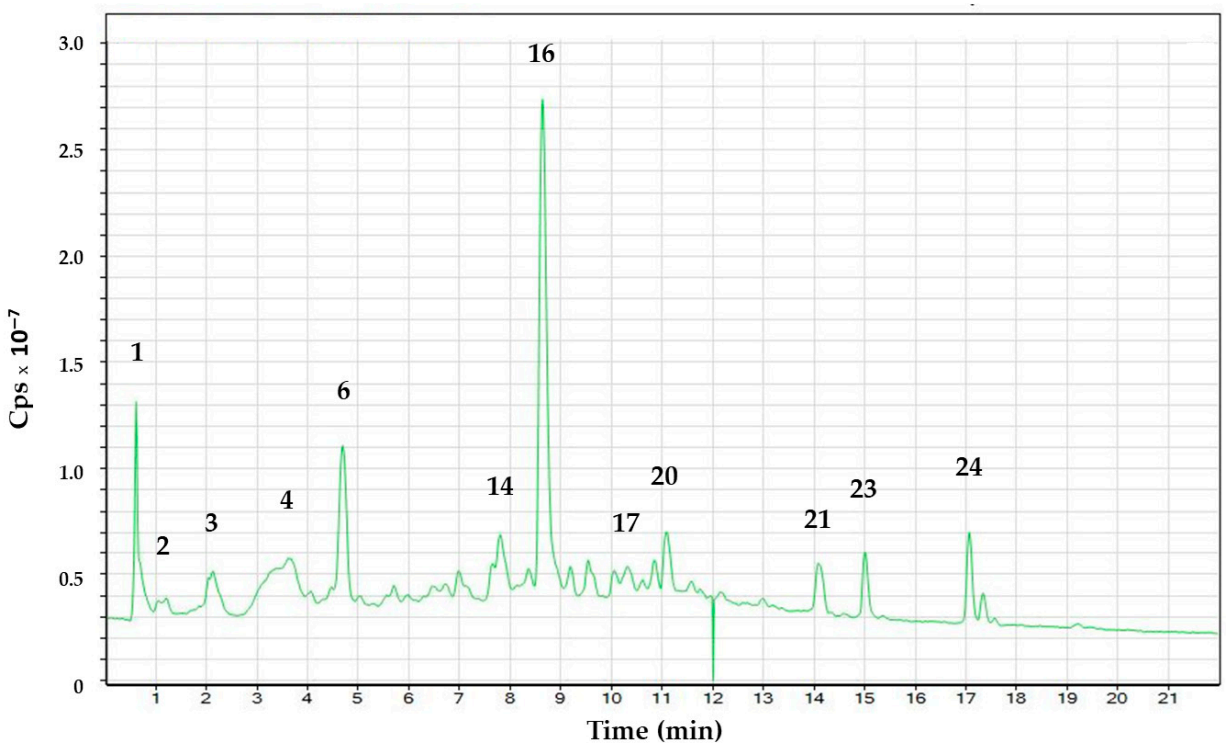


Figure 1. HPLC-MS chromatogram reporting the elution of the main chemical compounds present in the hydro-methanolic extract of *R. officinalis*. 1: quinic acid; 2: caffeic acid hexose I; 3: caffeic acid hexose II; 4: caffeic acid diglucoside; 6: coumaric acid apinosyl glucoside; 14: nepistrin; 16: rosmarinic acid; 17: luteolin 3(acetil) glucuronide I; 20: luteolin 3(acetil) glucuronide II; 21: cirsimaritin; 23: apigenin; 24: carnosol.

The HPLC-TOF-MS analysis determined several peaks corresponding to 24 compounds which were identified considering their retention time (Rt) and mass-to-charge ratio (m/z) (Figure 1). The chemical compounds that were relatively more abundant are displayed in Table 1.

Table 1. Tentative determination of compounds present in the hydromethanolic extracts (HME) of *R. officinalis* growing in Algeria by HPLC-TOF-MS and comparison with standards and literature.

Peak	Compound	Rt (min)	Molecular Formula Proposed $(M-H)^-$	*m/z* Experimental	*m/z* Calculated	Error (ppm)
1.	Quinic acid	0.62	$C_7H_{11}O_6$	191.05	191.05	−8.89
2.	Caffeic acid hexose I	2.04	$C_{15}H_{17}O_9$	341.09	341.08	−6.36
3.	Caffeic acid hexose II	2.10	$C_{15}H_{17}O_9$	341.09	341.08	−6.85
4.	Caffeic acid diglucoside	3.67	$C_{21}H_{29}O_{13}$	489.16	489.16	−4.12
5.	Sinapic acid hexoside	4.45	$C_{17}H_{21}O_{10}$	385.11	385.11	−5.03
6.	Coumaric acid apinosyl glucoside	4.51	$C_{20}H_{25}O_{12}$	457.13	457.13	−3.51
7.	Benzyl alcohol pentosylhexoside	4.60	$C_{18}H_{25}O_{10}$	401.14	401.14	−4.65
8.	Tuberonic acid glucoside (?)	4.70	$C_{18}H_{27}O_9$	387.16	387.16	−8.64
9.	Phloretin 2-xyloglucoside	5.69	$C_{26}H_{31}O_{14}$	567.17	567.19	−1.74
10.	Quercetin galactoside	5.93	$C_{21}H_{19}O_{12}$	463.08	463.08	−3.29
11.	Pterogynoside	6.99	$C_{29}H_{25}O_{14}$	597.12	597.12	−2.89
12.	Luteolin rutinoside	7.12	$C_{27}H_{29}O_{15}$	593.15	593.15	−2.13
13.	Luteolin glucoside	7.18	$C_{21}H_{19}O_{10}$	447.09	447.09	−3.12
14.	Nepistrin	7.80	$C_{22}H_{21}O_{12}$	477.10	477.10	−8.23
15.	Apigenin glucoside	8.33	$C_{21}H_{19}O_{10}$	431.10	431.09	−5.49
16.	Rosmarinic acid	8.64	$C_{18}H_{15}O_8$	359.07	359.07	−5.53
17.	Luteolin 3(acetil) glucuronide I	10.33	$C_{23}H_{19}O_{13}$	503.08	503.08	−5.53
18.	Luteolin	10.56	$C_{15}H_9O_6$	285.04	285.04	−5.16
19.	Feruloylnepitrin	10.61	$C_{31}H_{27}O_{14}$	623.14	623.14	−3.65
20.	Luteolin 3(acetil) glucuronide II	11.09	$C_{23}H_{19}O_{13}$	503.08	503.08	−4.96
21.	Cirsimaritin	14.07	$C_{17}H_{13}O_6$	313.07	313.07	−8.10
22.	6-Hydroxyluteolin 7,3′ dimethyl ether	12.98	$C_{17}H_{13}O_7$	329.06	329.06	−4.86
23.	Apigenin	15.01	$C_{15}H_8O_5$	268.03	268.03	−3.82
24.	Carnosol	17.06	$C_{20}H_{25}O_4$	329.17	329.17	−5.95

The current study identified several flavonoids and hydroxycinnamic acids in the sample under investigation. The flavonoids detected included feruloylnepitrin (*m/z* 623.14), pterogynoside (*m/z* 597.12), luteolin rutinoside (*m/z* 593.15), phloretin 2-xyloglucoside (*m/z* 567.19), luteolin 3 (acetil) glucuronide I (*m/z* 503.08), luteolin 3 (acetil) glucuronide II (*m/z* 503.08), and quercetin galactoside (*m/z* 463.08). Additionally, the hydroxycinnamic acids detected included caffeic acid (*m/z* 489.16), caffeic acid diglucoside (*m/z* 489.16), and coumaric acid apinosyl glucoside (*m/z* 457.13).

Rosemary or *R. officinalis* is a ubiquitous herbal tea that is widely consumed owing to its abundant phenolic compounds that have been associated with various health benefits. In this study, several flavonoids were identified, including feruloylnepitrin, pterogynoside, luteolin rutinoside, phloretin 2-xyloglucoside, luteolin 3 (acetil) glucuronide I and II, and quercetin galactoside, which were found to be abundant. Additionally, hydroxycinnamic acids such as caffeic acid, caffeic acid diglucoside, and coumaric acid apinosyl glucoside were also identified. These findings corroborate the results of a study by Achour et al. [33,34] on Tunisian rosemary tea, which reported high concentrations of the same components. Luteolin-7-glucoside and its derivatives (luteolin 3′-acetyl-O-glucuronide, luteolin-7-O-glucuronide, and Isorhamnetin-3-O-glucoside) were also found in high abundance in an Ortuño J. study [35]. Furthermore, rosmarinic acid and related compounds (rosmarinic acid-3-O-glucoside, rosmarinic acid-3-O-glucoside isomer, sagerinic acid) and carnosic acid were detected in high concentrations. Recently, Al-Khafaji and Al-Azawi [35] reported the identification of four important phenolic compounds (gallic acid, rosmarinic acid, ferulic acid, and caffeic acid) in rosemary extracts. These findings provide evidence of the abundance and diversity of phenolic compounds in rosemary, which may contribute to its health-promoting properties. Based on previous studies, it has been suggested that rosmarinic acid and carnosol are two of the most abundant phenolic compounds in extracts of *R. officinalis*, regardless of the part of the plant used for extraction or the country in which it was grown. Teruel et al. [35] observed high levels of these two compounds in extracts obtained from rosemary grown in Spain using different extraction methods. In contrast, *R. eriocalyx* extracts from Algeria were found to contain high amounts of carnosol, quercetin

galactoside, and rosmarinic acid [31]. These differences in chemical composition could be due to several factors, including the plant parts used for extraction, genotype, harvesting time, and experimental conditions such as the solvent, time of extraction, temperature, and technical procedures [36]. Nevertheless, the high levels of carnosol and rosmarinic acid in rosemary extracts suggest their potential use as natural preservatives for raw and processed meat [30,32,37].

3.2. CG-MS Analysis

The chemical composition of *R. officinalis* EO has been the focus of numerous studies, which have investigated samples collected from various locations and at different phenological stages, both in Algeria and around the world [38,39]. In this study, we examined the chemical composition of EO extracted from *R. officinalis* grown in Algeria. Table 2 summarizes the results of our analysis.

Table 2. Chemical composition of the essential oil from the aerial part of *R. officinalis* growing in Algeria.

N	Retention Index	Compound	%	S.E.	Identification by
1.	1010	α-Thujene	0.4	0.1	Chemical standard
2.	1015	3-carene	9.2	0.7	Chemical standard
3.	1021	2-Methylbicyclo [4.3.0] non-1(6)-ene	5.0	0.4	Nist 2014
4.	1087	ψ-Limonene	7.1	0.5	Chemical standard
5.	1113	β-Pinene	0.9	0.2	Chemical standard
6.	1182	p-Mentha-1,3-diene	0.5	0.1	Nist 2014
7.	1217	β-Cymene	0.6	0.1	Chemical standard
8.	1231	Eucalyptol	28.7	2.3	Chemical standard
9.	1251	gTerpinene	1.2	0.1	Chemical standard
10.	1263	(c/t)-Sabinene hydrate	0.7	0.1	Nist 2014
11.	1275	α-Terpinene	0.7	0.1	Chemical standard
12.	1291	(c/t)-Sabinene hydrate	0.3	0.1	Nist 2014
13.	1387	Linalyl formate	0.4	0.1	Nist 2014
14.	1489	Camphor	16.7	1.2	Chemical standard
15.	1586	Borneol	13.5	1.0	Chemical standard
16.	1594	Terpinen-4-ol	0.8	0.2	Chemical standard
17.	1602	α-Terpineol	3.4	0.3	Chemical standard
18.	1815	Bornyl acetate	5.8	0.4	Nist 2014
19.	1914	Bicyclo [5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	3.0	0.2	Nist 2014
20.	1943	1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene	0.3	0.1	Nist 2014
21.	2075	Sesquibenihiol	0.7	0.1	Nist 2014

In this analysis, twenty-one chemical compounds were identified in rosemary EO, with the main oxygenated monoterpene constituents being eucalyptol (28.7%), camphor (16.7%), and borneol (13.5%), while 3-carene (9.2%), ψ-Limonene (7.1%) and bornyl acetate (5.8%) were present in lower concentrations (Table 2, Figure 2). The composition of the *R. officinalis* samples collected in Northeast Algeria [16,40] was found to be similar to the present study, with eucalyptol (37.97% and 37.51%, respectively) and camphor (11.84% and 11.70%, respectively) being the major EO constituents. Another recent study on Tunisian *R. officinalis* EOs also found eucalyptol (37.56%) and camphor (7.10%) as the principal components [41]. Similarly, Rekioua [38] reported eucalyptol (37.51%) and camphor (11.70%) as the major chemical components in Algerian *R. officinalis* samples. Other studies have reported different levels of major components, with eucalyptol (25.5%), isobornyl acetate (15.7%), and camphor (12.2%) being the main components of EO obtained from *R. officinalis* grown in the United Arab Emirates [42], while two different EOs obtained from *R. officinalis* grown in Palestine contain three main components, namely eucalyptol (4.81–37.83%), α-pinene (13.07–51.36%), and camphor (11.95–24.30%) [43]. In contrast, there are only two major chemical components in EOs of *R. officinalis* from Brazil: α-pinene (26.26%) and bornyl acetate (17.83%) [44]. The differences in chemical composition could

be attributed to different ecotypes of the species, whether plants grow wild or cultivated, as well as environmental and ecological conditions, including climate, soil type, and agricultural practices. Sample processing techniques, drying, oil extraction method, and sampling period could also affect the oil's yield and chemical composition [45].

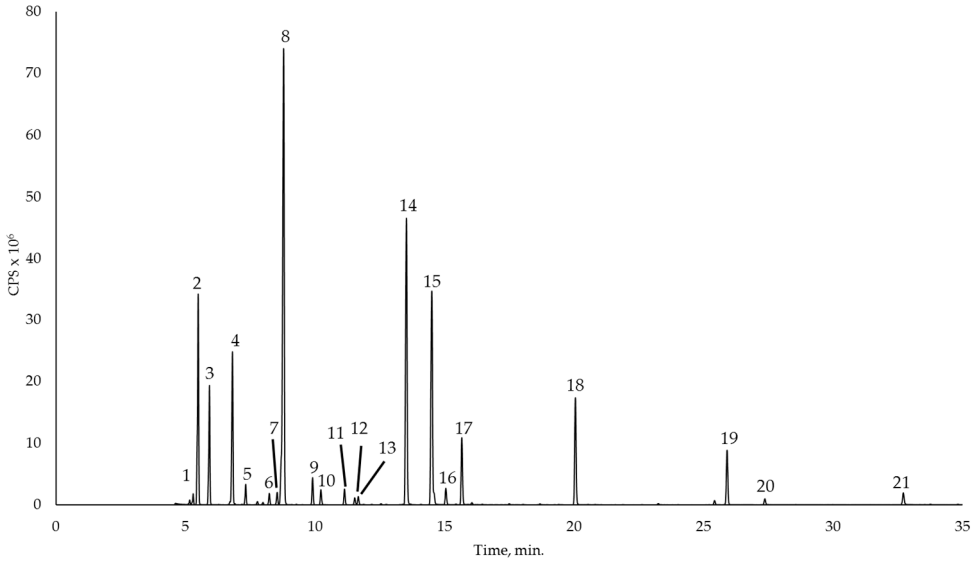


Figure 2. GC-MS chromatogram of essential oil of *R. officinalis* growing in Algeria. For the compound identification, see Table 2.

3.3. Antioxidant Activity

3.3.1. Total Polyphenol Content

Our study found a high level of total polyphenols in *R. officinalis* extract, with a content of 335.37 ± 9.33 µg gallic acid equivalent (GAE) per mg of extract. This result is consistent with previous studies by Boumadjen and Kimouche [45] and Kamli et al. [46], which reported high levels of polyphenols in *R. officinalis* extracts. However, our study detected a higher number of polyphenols than those reported by Dhouibi et al. [47] and Kabubii et al. [48] for Tunisian and Kenyan samples, respectively.

Among the bioactive compounds identified in *R. officinalis* leaves, rosmarinic acid, and carnosol were the main constituents, both of which are known for their antioxidant potential. These findings are significant, as oxidative stress is a key contributor to cognitive decline disorders, and the use of these phenolic compounds as therapeutic agents for the prevention and treatment of such diseases has been suggested [46].

The high content of phenolic components in *R. officinalis* extract is important for obtaining *R. officinalis* EO with high antioxidant activity. Additionally, the correlation between phenolic content and antioxidant activity suggests that the best time for harvesting the plant to obtain the highest number of phytochemicals is during the dry season or in specific agroecological zones [47–49].

To provide a clear visual representation of our findings, Table 3 summarizes the total polyphenol content and main bioactive constituents identified in rosemary samples from various studies.

Table 3. Total polyphenol content and main bioactive constituents identified in rosemary samples.

Study	Total Polyphenol Content (µg GAE/mg)
Current study	335.37 ± 9.33
Boumadjen and Kimouche [45]	248.55
Kamli et al. [46]	804 (ethyl-acetate), 473 (ethanol), 273 (water)
Dhouibi et al. [47]	85.27
Kabubii et al. [48]	39.71

3.3.2. Comparative Evaluation of In-Vitro Antioxidant Activity Using DPPH, ABTS, FRAP Galvinoxyl scavenging assay and CUPRAC assays.

The antioxidant capacity of *R. officinalis* EO's, was evaluated using multiple assays, including DPPH radical scavenging, ABTS reducing power, FRAP-reducing power, galvinoxyl scavenging, and cupric ion reducing antioxidant capacity (CUPRAC) assays. A summary of the results is presented in Table 3.

HME: hydro-methanolic extract; EO: essential oil; AChE: Acetylcholinesterase; BChE: Butyrylcholinesterase; IC_{50}: The concentration giving a reduction of 50%; na: not active; BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene.

From the data presented in Table 4, it can be observed that *R. officinalis* HME showed a high level of antioxidant activity (IC50), while EO showed a low level of antioxidant activity in the ABTS test (IC50: 667.09) and had no activity in the other test. In particular, HME showed significant clearance activity when assessed by DPPH, ABTS and GOR assays with IC50 values ranging from 36 to 40 $\mu g \cdot mL^{-1}$. In addition, BHT and BHA tests showed that HME had relatively higher scavenging activity compared with DPPH and ABTS, with IC50 values of 6.82 and 1.59 $\mu g \cdot mL^{-1}$, respectively (Table 3). Furthermore, *R. officinalis* HME showed higher antioxidant capacity, as measured by the CUPRAC assay, in contrast to EO, which showed no activity. Polyphenols, including the rosmarinic acid present in *Rosmarinus officinalis* extracts, have the ability to scavenge free radicals. They can donate hydrogen atoms or electrons to unstable radicals, neutralizing them and preventing them from causing oxidative damage to cells. The polyphenols act as potent antioxidants by intercepting and quenching free radicals. These outcomes may be attributed to the nature of the components, their synergism, and their mechanism's activity. However, it should be noted that the results obtained were still lower than those achieved with the BHA (9.62 $\mu g \cdot mL^{-1}$) and BHT (3.64 $\mu g \cdot mL^{-1}$) standards.

Table 4. Antioxidant activity of *R. officinalis* EOs and HME: comparison and significance analysis (n = 3, log-transformed data, Tukey's HSD Test: p-Value < 0.01 (^{a,b} and ^{b,c}); p-Value < 0.001 (^{a,c})).

	DPPH IC_{50}, $\mu g \cdot mL^{-1}$	**ABTS IC_{50}, $\mu g \cdot mL^{-1}$**	**FRAP A0.5, $\mu g \cdot mL^{-1}$**	**Galvinoxyl Scavenging Assay EC_{50}, $\mu g \cdot mL^{-1}$**	**CUPRAC A0.5, $\mu g \cdot mL^{-1}$**
HME	40.44 ± 0.81 $^{(a)}$	39.02 ± 1.02 $^{(a)}$	72.40 + 2.89 $^{(a)}$	36.07 + 0.45 $^{(a)}$	31.36 + 1.36 $^{(a)}$
EO	na	667.09 ± 3.36 $^{(b)}$	Na	na	na
BHA/HME	6.82 ± 0.49 $^{(b)}$	1.59 ± 0.03 $^{(c)}$	-	3.32 ± 0.18 $^{(b)}$	9.62 ± 0.87 $^{(b)}$
BHT/HME	6.82 ± 0.49 $^{(b)}$	1.03 ± 0.00 $^{(c)}$	-	5.38 ± 0.06 $^{(b)}$	3.64 ± 0.19 $^{(b)}$
BHA/EO	6.14 ± 0.41 $^{(b)}$	1.29 ± 0.30 $^{(c)}$	-	3.32 ± 0.18 $^{(b)}$	5.35 ± 0.71 $^{(b)}$
BHT/EO	12.99 ± 0.41 $^{(c)}$	1.81 ± 0.10 $^{(c)}$	-	5.38 ± 0.06 $^{(b)}$	8.97 ± 3.94 $^{(b)}$
α-Tocopherol	13.02 ± 5.17 $^{(c)}$	-	34.93 ± 2.38 $^{(b)}$	-	-
Ascorbic acid	-	-	6.77 + 1.15 $^{(c)}$	-	-
Tannic acid	-	-	5.39 + 2.38 $^{(c)}$	-	-

In Table 4, the HME of *R. officinalis* exhibited a reducing power from ferric to ferrous iron. However, the reducing power was lower when compared to the reference compounds of ascorbic acid, tannic acid, and α-tocopherol. The IC50 values of the samples were lower than the standards BHA, BHT, α-tocopherol, ascorbic acid, and tannic acid for all tests conducted. The extract showed a significant percentage of inhibition, indicating that polyphenols, including rosmarinic acid, have the ability to chelate metal ions such as iron (Fe) through their hydroxyl groups. The hydroxyl groups in polyphenols act as ligands, forming coordination bonds with the metal ions. This process leads to the formation of stable complexes between the polyphenols and the metal ions. The hydroxyl groups in them can donate electrons to the metal ions, forming coordinate covalent bonds. This chelation prevents the metal ions from participating in oxidative reactions, where these metal ions can generate reactive oxygen species (ROS) such as hydroxyl radicals (OH•). By chelating the metal ions, polyphenols effectively sequester them and reduce their ability to catalyze

the production of ROS. This chelation activity helps prevent oxidative damage caused by the metal ions and contributes to the antioxidant properties of polyphenols. [50,51].

Regarding *R. officinalis* EO activity, the results of the study showed that the rosemary EO had lower ABTS inhibition activity compared to BHA and BHT at all tested concentrations. The A0.50 values, which represent the concentration required to inhibit 50% of the ABTS radicals, were much higher for EO compared to BHA and BHT. The findings of this study were lower than those reported by Fadili et al. [50], who found an IC_{50} of 0.05 mg·mL^{-1} for the methanolic extract of *R. officinalis*.

A clear relationship between the polyphenols' concentration and antioxidant activity, confirming that rosmarinic acid's polyphenols are potent antioxidants able to inhibit the production of free radicals and prevent the oxidation of macromolecules [52]. The antioxidant activity of the HME is depending on the type and structure of the antioxidants present, as well as the quantity of polyphenols.

The reducing capacity test, according to Huang et al. [50], is an electron transfer-based assay that measures the reductants (antioxidants) preexisting in a sample with the aid of a spectrophotometric redox reaction [51]. Based on previous studies, it can be inferred that the primary chemicals responsible for the extract's reducing potential are polyphenols (Table 3) [52]. These compounds are well-qualified as outstanding electron donors, which enables them to neutralize free radicals and behave as chain-breaking antioxidants. Furthermore, they can interact with peroxide precursors and thus inhibit peroxide production.

According to Bendary et al. [53], polyphenols are frequently acknowledged to be exceptional electron donors, which enables them to neutralize free radicals and act as chain-breaking antioxidants. In addition, these compounds may also interact with precursors of peroxide and cause the inhibition of its production. The reducing ability of our samples is caused by the reductants' presence, which serves as electron donors [54]. Because of their capacity to transfer electrons, they may also restore diverse oxidized antioxidants such as vitamin E [55]. The choice of solvent is crucial for the extraction of phenols and contributes to their effectiveness as antioxidants. A recent study conducted on *R. tournefortii* de Noé from Algeria by Bensouici et al. [56] revealed that butanol extracts exhibited the highest antioxidant activity in tests such as DPPH, ABTS, O_2-DMSO alkaline, reducing power, β-Carotene-linoleic acid, and CUPRAC assays. The same authors noted that the essential oil was only effective in chelating ferrous ions in the β-Carotene linoleic acid assay and did not show any scavenging activity. The ferric-reducing capacity of compounds is measured by their ability to donate a hydrogen atom to the ferric complex and break the radical chain reaction, thereby preventing oxidative damage by directly scavenging free radicals. On the other hand, the ferrous ion chelating capacity of the compounds measures their ability to chelate metal ions, thus indirectly preventing the generation of hydroxyl radicals via the Fenton reaction. It is noteworthy that essential oils with a strong ferrous ion chelating (FIC) capacity usually exhibit moderate or low primary antioxidant activity [57]. It is well documented that the potent antioxidant activity of rosemary extract is mostly attributed to its content of rosmarinic acid, carnosol, and carnosic acid [58]. In addition, rosmadial, rosmanol, rosmarinic acid, carnosol, and carnosic acid were detected as major antioxidant components of rosemary extract. However, carnosic acid, which is recognized as the most effective antioxidant constituent, is known to be unstable in the presence of oxygen, leading to the formation of new compounds through the decomposition of carnosic acid, primarily carnosol, and rosmanol [36]. On the other hand, the radical scavenging ability of *R. officinalis* extracts has been reported to be related to their chemical constituents. Phenolics and flavonoids, particularly chlorogenic acid and luteolin derivatives, have been shown to be efficient scavengers. With respect to chelation activity, which involves the creation of a complex with hetero-atoms, chlorogenic acid has exhibited a high chelating effect [59].

3.4. Anticholinesterase Enzyme's Inhibitory Activity

The anticholinesterase activity of *R. officinalis* EO and HME was evaluated using a combination of two complementary methods: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) assays (Table 5). The assay of AChE inhibitory activity demonstrated that *R. officinalis* EO exhibited efficient inhibition (70.31 µg mL^{-1}); however, its inhibitory activity was lower than the galantamine standard (6.27 µg mL^{-1}). Conversely, *R. officinalis* HME did not exhibit inhibitory activity on AChE, even at different increasing concentrations. The results for BChE inhibitory activity revealed that neither EO nor HME was capable of inhibiting BChE.

Table 5. Enzyme inhibitory activity of *R. officinalis* Essential oil (EO) and hydro-methanolic extract HME.

	AChE	**BChE**	**α-Glucosidase**	**Tyrosinase**
	IC_{50} µg mL^{-1}			
HME	na	na	na	na
EO	70.31 ± 5.00	na	na	na
Galantamine	6.27 ± 1.15		-	-
Quercetin	-		4.26 ± 0.24	-
Kojic acid	-		-	25.23 ± 0.78

HME: hydro-methanolic extract; EO: essential oil; AChE: Acetylcholinesterase; BChE: butyrylcholinesterase; BHA: butylated hydroxyanisol, BHT: betahydroxytoluene; IC_{50}: the concentration causing a reduction of 50%; na: not active.

The results are consistent with those of Bensouici et al. [56] who reported that *R. officinalis* essential oil is a potentially potent inhibitor of AChE, while chloroform and butanolic extracts are less effective AChE inhibitors. Additionally, these authors observed that *R. officinalis* EO also inhibits BChE, which was not observed in the current study.

3.4.1. α-Glucosidase Inhibition Activity

In the α-glucosidase inhibitory test, which evaluates the activity of the enzyme responsible for the breakdown of glucose, both HME and EO showed no inhibitory activity (Table 4).

In contrast to our study, another investigation presented that the diethyl ether fraction of *R officinalis* exhibited potent α-glucosidase inhibition, which was higher than that of the acarbose standard. The same study reported that the level of inhibitory activity for α-glucosidase was dose-dependent [60]. These results are consistent with those obtained from the inhibitory activity assay of the chloroform extract of *R. tournefortii* [56].

In the context of diabetes, α-glucosidase is a crucial enzyme that catalyzes the breakdown of maltose or sucrose, leading to abnormal blood glucose levels. Therefore, inhibitors of α-glucosidase could potentially reduce abnormally high plasma glucose levels following carbohydrate consumption [60].

It has been noted that chlorogenic acid exhibited a combined inhibitory activity on porcine pancreatic α-amylase, which could be due to the high level of apigenin and its derivatives, which have a great inhibitory ability on digestive enzymes [61].

Regarding *R. officinalis* EO, the oxygenated monoterpenes, namely, eucalyptol and α-terpineol, demonstrated a synergistic effect on α-glucosidase inhibitory activity. The presence of other compounds such as camphor, borneol, linalool, and linalyl acetate conferred great potential in combination. However, they exhibited weak inhibitory activity when evaluated separately [62].

On the other hand, in vivo analysis demonstrated that the butanolic and diethyl ether fractions of *R. officinalis* decreased the α-glucosidase activity in the mice intestines. The antihyperglycemic effect exhibited by the butanolic fraction could be attributed to the presence of flavonoids, which have been previously reported to possess anti-diabetic

properties. Therefore, the flavonoid-rich fractions of *R. officinalis* exert an α-glucosidase inhibition and antihyperglycemic effect by inhibiting glucose transport, resulting in a decrease in blood glucose levels [60].

3.4.2. Tyrosinase Inhibition Activity

The anti-tyrosinase activity of *R. officinalis* EO and HME was found to be weak in the photo-protective assay, as the tested concentrations were not able to inhibit 50% of the enzymatic activity (Table 5). Although there are few studies on the tyrosinase inhibitory activity of rosemary, recent research has emphasized the significance of its bioactive compounds in promoting treatment against UV light exposure, atopic dermatitis, and pollution-induced skin aging in both in vitro and in vivo studies [63].

The antioxidant potential of a rosemary extract has been confirmed by Ezzat et al. [64], who identified rosmarinic acid, carnosic acid, carnosol, and rosmanol as the key compounds responsible for the extract's anti-wrinkle action. The topical application of antioxidants has been recognized as an effective approach to reducing skin damage caused by photoaging by protecting cells and tissues from free radicals.

In vitro sun protection factor (SPF) evaluation and in vivo animal model testing of the *R. officinalis* extract (emulgel) has shown their protective effect against tissue damage resulting from UVB radiation. The synergistic antioxidant and photoprotective ability of rosemary extract can be attributed to its main constituents, particularly rosmarinic acid, carnosic acid, and carnosol [65].

3.4.3. Evaluation of Limit of Detection, and Limit of Quantification

The aims of utilizing the limit of detection (LOD) and limit of quantitation (LOQ) in analytical chemistry are twofold. The LOD serves to determine the lowest concentration at which an analyte can be reliably detected above the background noise level [66]. This sensitivity assessment is particularly valuable for identifying the presence or absence of analytes in samples with low-level concentrations or high background interference. On the other hand, the LOQ establishes the lowest concentration at which an analyte can be quantified with acceptable precision and accuracy. By ensuring accurate quantification within a defined confidence range, the LOQ enables reliable measurements and the assessment of analyte concentrations in various samples.

The determination of the limits of detection (LOD) and limits of quantification (LOQ) is essential in assessing the sensitivity and reliability of the biological assays employed in this study. The results obtained demonstrated that the assays used in this study are reliable and reproducible. Table 6 presents the values of LOD and LOQ obtained for each assay. These results are important for future studies using the same methods, as they provide a reference for the lowest amount of analyte that can be detected or quantified with confidence.

Table 6. The LOD and LOQ values for the biological assays of *R. officinalis* EO and HME.

Extract	Test	LOD μg mL^{-1}	LOQ μg mL^{-1}
HME	TPC	30.75	93.3
BHA	DPPH	36.50	115.64
BHT	DPPH	38.08	120.62
α-Tocopherol	DPPH	43.55	137.82
HME	ABTS	1.56	3.12
BHT	ABTS	6.25	25
BHA	ABTS	25	50
HME	CUPRAC	0	0
BHA	CUPRAC	0.05	0.15
BHT	CUPRAC	0.07	0.21
EO	ABTS	3.06	10.20
BHA	ABTS	0.05	0.16
BHT	ABTS	0.01	0.03
EO	GOR	-	-
BHT	GOR	12.22	36.92
BHA	GOR	47.68	144.60
MER	GOR	0.18	0.55
BHT	GOR	0.59	1.78
BHA	GOR	0.58	1.59
HME	FRAP	0.02	0.09
Ascorbic acid	FRAP	0.10	0.34
Tannic acid	FRAP	0.12	0.41
α-Tocopherol	FRAP	0.03	0.13

LOD: limit of detection; LOQ: limit of quantification; HME: hydromethanolic extract: EO: essential oil; DPPH: 2,2-diphenyl-1-picrylhydrazyl; EHMR: methanolic extract of rosemary; BHA: butylated hydroxyanisol; BHT: butylated hydroxytoluene.

4. Conclusions

The study conducted aimed to analyze the qualitative and quantitative composition of the essential oil (EO) and hydroethanolic extract (HME) obtained from *Rosmarinus officinalis* cultivated in Algeria. The results of the investigation revealed that both the EO and HME contained a high concentration of potent compounds with antioxidant activity. Notably, the HME was found to be rich in rosmarinic acid and carnosol, indicating its potential as a natural food preservative. On the other hand, the main components of the EO were eucalyptol, camphor, and borneol. These findings align with previous reports that have also highlighted the strong antioxidant activity of *R. officinalis* extracts. The observed inhibitory effects of both the EO and HME on enzymatic activity are noteworthy. However, further research is required to determine their efficacy at higher doses while ensuring food safety. Future studies could include in vivo toxicity tests and in silico modeling to evaluate the potential benefits and risks associated with different doses of *R. officinalis* extracts. In summary, this study demonstrates that the EO and HME obtained from *R. officinalis* cultivated in Algeria are rich in bioactive compounds with potent antioxidant properties. These findings suggest potential applications of rosemary (R. officinalis) extracts in the food industry. The study contributes to the growing body of evidence supporting the health-promoting effects of *R. officinalis* extracts

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