Send Orders for Reprints to reprints@benthamscience.net

The Natural Products Journal, **2020,** *10,* **1-11**

RESEARCH ARTICLE

LC-MS/MS Profiling of 37 Fingerprint Phytochemicals in *Oenanthe fistulosa* L. and its Biological Activities

Nabila Souilah[1,2], Hamdi Bendif[3,4,*], Zain Ullah[5], Mohamed Djamel Miara[6], Messaoud Laib[2], Mehmet Öztürk[5], Salah Akkal[1], Kamel Medjroubi[1] and Ahmed M. Mustafa[7,*]

[1]Department of Chemistry, Faculty of Exact Sciences, University of Constantine 1, Constantine, Algeria; [2]Department of Natural and life Sciences, Faculty of Sciences, University of Skikda, Skikda, Algeria; [3]Department of Natural and Life Sciences, Faculty of Sciences, University of M'sila, BP 166 M'sila, 28000, Algeria; [4]Laboratory of Ethnobotany and Natural Substances, Department of Natural Sciences, Ecole Normale Supérieure (ENS), Kouba, BP 92 Kouba 16308, Algeria; [5]Department of Chemistry, Muğla Sitki Koçman University, Kötekli-48000, Muğla, Turkey; [6]Department of Biology, University of Tiaret 14000. DZ, Tiaret, Algeria; [7]Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig–44519, Egypt

ARTICLE HISTORY

Received: July 20, 2019
Revised: September 14, 2019
Accepted: October 23, 2019

DOI:
10.2174/2210315509666191111102557

Abstract: ***Introduction*:** *Oenanthe fistulosa* L. (Apiaceae) is often associated with damp soils. Its underground parts and the young leaves are mainly cooked with other vegetables.

***Objective*:** The aim of the current work was to investigate the chemical profile of dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BuOH) fractions of *O. fistulosa* through analysis of 37 phytochemicals by LC-MS/MS and to evaluate their biological activities such as antioxidant, anticholinesterase and antityrosinase for the first time.

***Methods*:** Analysis of 37 phytochemicals was performed by liquid chromatography-mass spectrometry (LC-MS/MS). Antioxidant activity was evaluated using five *in vitro* assays, while anticholinesterase and anti-tyrosinase activities were performed using Ellman and Dopachrome methods, respectively.

***Results*:** The number of phenolic compounds detected in DCM, EA and BuOH fractions was found to be 9, 15, and 12; respectively. More specifically, 9 phenolic acids were detected and among them, chlorogenic, *tr*-ferulic and *p*-coumaric acids were the most abundant. While 8 flavonoids were detected and apigetrin, rutin, and quercitrin were the most abundant. In addition, 3 non-phenolic organic acids (quinic, malic and fumaric acids) were detected in large quantities. Furthermore, the tested plant fractions demonstrated a noteworthy and strong antioxidant action. The plant displayed very strong action against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes; and BuOH fraction was the most potent one. Finally, BuOH and DCM fractions showed good tyrosinase inhibitory activity.

***Conclusion*:** According to the obtained results, *O. fistulosa* might be a promising candidate for the alleviation of oxidative stress, neurodegenerative (such as Alzheimer's disease) and hyperpigmentation disorders.

Keywords: *Oenanthe fistulosa*, apiaceae, polyhenolics, LC-MS/MS, antioxidant, anticholinesterase, antityrosinase.

1. INTRODUCTION

The genus *Oenanthe* (Apiaceae) is represented by aquatic plants which are perennial, hemicryptophyte, sometimes helophyte, 30 to 100 cm high. It includes 40 species distributed in the temperate northern hemisphere, Europe, western Asia, India and northern Africa [1]. The name *Oenanthe* signifies "wine flower", because the plant produces a state of stupefaction similar to drunkenness. This, as well as locked jaws (*risus sardonicus*) has been documented in human poisoning from *O. crocata*, a plant that is common only in Sardinia within the Mediterranean area [2].

Oenanthe fistulosa L. *(common name:* Tubular waterdropwort) is an erect perennial, glabrous umbelliferous herb. It is often associated with damp soils and still widespread but declined across much of southern England, Ireland and coastal regions of Wales. It is a rare species in Scotland and is assessed as Vulnerable in Great Britain as a whole [3].

*Address correspondence to these authors at the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig–44519, Egypt; E-mails: a_m_elkadeem@zu.edu.eg, ahmed.mustafa@unicam.it
Department of Natural and Life Sciences, Faculty of Sciences, University of M'sila, BP 166 M'sila, 28000, Algeria; E-mail: bendif_hamdi@yahoo.fr

2210-3155/20 $65.00+.00

Phytochemical assessments have revealed that the genus *Oenanthe* contains various bioactive compounds such as essential oils, polyacetylenes, bitter principles, coumarins, flavonoids, flavonoid glycosides and polyphenols [2, 4]. For example, *O. fistulosa* and *O. crocata* have been reported to have polyacetylene toxins and bitter principles. An investigation of *Oenanthe fistulosa* from Sardinia afforded oenanthotoxin and dihydrooenanthotoxin from the roots and the diacetylenic epoxydiol from the seeds [2].

A variety of biological activities of the genus *Oenanthe* has been reported [2, 4]. Oenanthotoxin and dihydrooenanthotoxin isolated from *Oenanthe fistulosa* were found to potently block GABAergic responses leading to neurotoxic activity and providing a molecular rationale for the symptoms of poisoning from water-dropwort (*Oenanthe crocata*) and related plants [2]. The essential oil of *Oenanthe crocata* was reported to have antifungal, antioxidant and anti-inflammatory activities [5].

It is noteworthy that five informants reported the use of the underground parts and the young leaves of *Oenanthe fistulosa* as food, which was mainly cooked with other vegetables. The use of this species is new, possibly because the plant may be toxic, although the means of preparation might reduce its toxicity [6].

The aim of the current work was to investigate the chemical profile of *O. fistulosa* through identification and quantitation of the phenolic compounds by LC-MS/MS and to evaluate their biological activities such as antioxidant, anticholinesterase and antityrosinase. The present study is a trial to focus on and discover the health benefits of this forgotten plant, hoping to lead us to the development of functional food ingredients for the prevention and treatment of various diseases such as oxidative stress, neurodegenerative and hyperpigmentation disorders.

2. MATERIALS AND METHODS

2.1. Chemicals and Materials

2.1.1. For LC-MS/MS Analysis

The analytical standards, HPLC-grade ammonium formate, acetonitrile and formic acid were purchased from Sigma–Aldrich (Milano, Italy).

2.1.2. For Biological Studies

Quercetin, potassium persulfate, ferrous chloride, ferric chloride, pyrocatechol, quercetin, copper (II) chloride, ethylenediaminetetraacetic acid (EDTA) and boron trifluoride-methanol complex (BF_3:MeOH) were obtained from E. Merck (Darmstadt, Germany). *β*-Carotene, linoleic acid, polyoxyethylenesorbitane monopalmitate (Tween-40), Folin–Ciocalteu's reagent, 3-(2-pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5',5''-disulfonic acid disodium salt (Ferene), neocuproine and ammonium acetate, butylhydroxytoluene (BHT), 2,2- diphenyl-1-picrylhydrazyl (DPPH) dye, Electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U/mg), acetylthiocholine iodide, horse serum butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4 U/mg), butyrylthiocholine chloride, 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) and galantamine were purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). 2,20-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was obtained from Fluka Chemie (Fluka Chemie GmbH, Sternheim, Germany). All other reagents, unless indicated were purchased from Sigma (St. Louis, MO, USA). Analytical grade reagents and solvents were consumed throughout the work.

2.2. Plant Material and Extraction Method

The aerial parts of *O. fistulosa* were collected in May 2015 in a full bloom state from El Kala, province of El Taref (North-East Algeria, -5 to +1200 m above sea level, 36.8905° N, 8.4451° E). The species was identified by the Forest Engineer A. Gurira (El Kala National Park). A voucher (ChifaDZUMCAPBC000040) was deposited in the Herbarium El Kala National Park, Algeria.

The vegetal material (aerial parts) was dried at room temperature in shade for one week and powdered. After, it (500 g) was exhaustively extracted by maceration in a mixture of methanol/water (70/30, v/v) at a ratio of 1:10 (w/v) for 24 h with constant stirring (speed of 200 rpm) and at room temperature. The solvents were evaporated at 40 °C using a Rotavapor (Büchi R-200, Germany) to afford 17.68 g extract. The crude extract was dissolved in 90 % aqueous methanol and fractioned with different solvents. The first fraction was carried out with 100 ml (3×) of dichloromethane (DCM), which was evaporated under reduced pressure to give a semisolid residue. This process was repeated with ethyl acetate (EA) and n-butanol (BuOH). The yields of DCM, EA and BuOH fractions were 0.70, 0.41, and 1.11 %, respectively. After, each fraction was dissolved in methanol and kept at 4 °C for its further analysis.

2.3. Preparation of Standards

Standard stock solutions were prepared in methanol (50 µg/ml) except hesperidin and isoquercitrin, that were dissolved in dimethylsulfoxide (50 µg/ml). Working solutions were prepared from the stock solutions by dilution in methanol. All solutions were stored in a refrigerator at 4 °C until analysis.

2.4. LC-MS/MS Analysis

2.4.1. Sample Preparation for LC-MS/MS

Samples of each fraction were diluted to 1000 mg/L and filtered with a 0.2 µm syringe filter prior to LC-MS/MS analysis.

2.4.2. Chromatographic Instruments and Conditions for LC-MS/MS

The quantitative study of 37 phytochemicals was performed using a Nexera Shimadzu UHPLC model coupled to an MS tandem instrument [7]. The chromatograph was equipped with LC-30AD binary pumps, a CTO-10ASvp column oven, a DGU-20A3R degasser and a SIL-30AC autosampler. Chromatographic separation was performed on an RP-C18 Insertil ODS-4 analytical column (100 mm x 2.1 mm, 2 µm). The temperature of the column was set at 35 °C. The elution gradient consisted of eluent A (water, 10 mM

ammonium formate and 0.1% formic acid) and eluent B (acetonitrile). The following gradient elution program was applied: 5-20% B (0-10 min), 20% B (10-22 min), 20-50% B (22-36 min), 95% B (36-40 min), 5% B (40-50 min). The solvent flow rate was maintained at 0.25 mL/min and the injection volume was adjusted to 4 µl.

MS detection was performed using a Shimadzu LC-MS brand 8040 tandem mass spectrometer model equipped with an ESI source operating in negative ion mode. LC-ESI-MS/MS data was collected and processed by LabSolutions software (Shimadzu). The multiple reaction monitoring (MRM) mode was used to quantify the analytes. The working conditions of the mass spectrometer were the following: interface temperature, 350 °C; DL temperature, 250 °C; temperature of the thermal block, 400 °C; nebulization gas flow (nitrogen), 3 L/min; and drying gas stream (nitrogen), 15 L/min.

2.5. Quantification of Total Phenolic Content

The total phenolic content was evaluated according to the method mentioned by Djeridane *et al*. [8]. The results were expressed in mg gallic acid equivalent (GAE) per g dry extract.

2.6. Quantification of Total Flavonoid Content

The total content of flavonoids was determined according to the method described by Djeridane *et al.* [8] and the concentrations were expressed in mg quercetin equivalent (QE) per g dry extract.

2.7. Biological Activities

2.7.1. Evaluation of Antioxidant activity by β-carotene Bleaching Test

The antioxidant activity of the extracts was evaluated using the *β*-carotene-linoleic acid system [9, 10]. The bleaching rate (R) of *β*-carotene was determined from the following equation:

$$R = \ln_{a/b} / t.$$

Where ln is the natural log, a is the absorbance at zero time, b is the absorbance at time t (120 min). The antioxidant activity as percent was calculated by the following equation:

Antioxidant activity (%) = (R control - R sample / R control) x 100.

Quercetin, catechin, BHT and *α*-tocopherol were used as antioxidant standards.

2.7.2. DPPH Free Radical Scavenging Test

The free radical scavenging activity of the extracts was determined by the test described by Öztürk *et al*. [9, 11]. The DPPH radical scavenging effect as percent was calculated from the following equation:

Antioxidant activity (%) = [A control - A sample / A control] × 100.

Quercetin, catechin, BHT and *α*-tocopherol antioxidant were used as standards.

2.7.3. ABTS Radical Cation Reduction Test

Reducing the power of the studied extracts using the $ABTS^{\cdot+}$ radical cation was determined according to the method of Re *et al*. [12] with slight modification. Free radical scavenging activity-was calculated using the equation:

$ABTS^{\cdot+}$ scanning activity (%) = [A control - A sample / A control] × 100

Quercetin, catechin, BHT and *α*-tocopherol were used as standards.

2.7.4. Total Antioxidant Capacity Test

The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method described by Ramalakshmi *et al*. [13] and expressed by the following equation:

Total antioxidant activity (%) = (1- absorbance of sample/absorbance of control) x 100

Quercetin and ascorbic acid antioxidant were used as standards.

2.7.5. Cupric Reducing Antioxidant Capacity (CUPRAC) Test

The cupric ion reducing capability was determined according to the method described by Apak *et al*. [14] with slight modification. The results were given as $A_{0.50}$ (µg/ml) corresponding to the concentration indicating 0.50 absorbances. The antioxidant BHT and *α*-tocopherol were used as standards.

2.7.6. Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activities

The inhibitory activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were evaluated by the method described by Öztürk *et al.* [9, 15]. The percent inhibition of AChE or BChE was obtained using the formula:

$$\% \text{ inhibition} = (E - S) / E\text{-}100$$

Where E is the enzyme activity without the test extract, and S is the enzyme activity with the tested extract. The assays were performed in triplicated and galantamine was used as a reference compound.

2.7.7. Tyrosinase Inhibitory Activity

The tyrosinase inhibitory activity of the extracts relative to kojic acid and L-mimosine standards was determined using fungal tyrosinase, according to Khatib *et al*. [16]. The percent inhibition of the enzyme was calculated according to the following formula:

$$\% \text{ inhibition} = [A - B /A] \times 100$$

Here, A and B are the absorbances of the control and samples; respectively.

2.8. Statistical Analysis

All data of antioxidant, anticholinesterase and antityrosinase activity tests were the average of three analyses. The data were recorded as mean ± standard deviation.

Table 1. HPLC–MS/MS acquisition parameters used for the analysis of the 37 marker compounds in the fractions of *O. fistulosa*.

No.	Compounds	Retention Time (min)	Scan type	Polarity or (ESI Mode)	Precursor ion [M-H]$^-$ (m/z)	MS2 Fragments or Product Ions (m/z)
1	Quinic acid	1.13	MRM	Negative	190.95	85.3-93.3
2	Malic acid	1.23	MRM	Negative	133.00	115.2-71.3
3	Fumaric acid	1.48	MRM	Negative	115.00	71.4
4	Gallic acid	3.00	MRM	Negative	168.85	125.2-79.2
5	Protocatechic acid	4.93	MRM	Negative	152.95	108.3
6	Pyrocatechol	6.48	MRM	Negative	109.00	108.35-91.3
7	Chlorogenic acid	7.13	MRM	Negative	353.15	191.2
8	*p*-Hydroxybenzoic acid	7.39	MRM	Negative	136.95	93.3-65.3
9	Vanillic acid	8.57	MRM	Negative	166.90	152.3-108.3
10	Caffeic acid	8.80	MRM	Negative	178.95	135.2-134.3
11	Syringic acid	9.02	MRM	Negative	196.95	182.2-167.3
12	Vanillin	10.87	MRM	Negative	151.00	1363-92.2
13	Salicylic acid	11.16	MRM	Negative	136.95	93.3-65.3
14	p-Coumaric acid	11.53	MRM	Negative	162.95	119.3-93.3
15	Rutin	12.61	MRM	Negative	609.05	300.1-271.1
16	*tr*-Ferulic acid	12.62	MRM	Negative	192.95	178.3
17	Sinapic acid	12.66	MRM	Negative	222.95	208.3-149.2
18	Hesperidin	12.67	MRM	Negative	609.00	301.1
19	Isoquercitrin	13.42	MRM	Negative	463.00	300.1-271.1
20	Rosmarinic acid	14.54	MRM	Negative	359.00	161.2-197.2
21	Nicotiflorin	14.68	MRM	Negative	593.05	285.1-255.2
22	α-Coumaric acid	15.45	MRM	Negative	162.95	119.4-93.3
23	Rhoifolin	16.11	MRM	Negative	577.05	269.2-211.1
24	Quercitrin	16.41	MRM	Negative	447.15	301.1-255.1
25	Apigetrin	16.59	MRM	Negative	431.00	268.2-239.2
26	Coumarin	17.40	MRM	Negative	147.05	91.0-103.2
27	Myricetin	18.72	MRM	Negative	317.00	179.2-151.3
28	Fisetin	19.30	MRM	Negative	284.95	135.2-121.3
29	Cinnamic acid	25.61	MRM	Negative	147.00	103.15-77.3
30	Liquiritigenin	25.62	MRM	Negative	254.95	119.3-135.1
31	Quercetin	28.17	MRM	Negative	300.90	151.2-179.2
32	Luteolin	28.27	MRM	Negative	284.75	133.2-151.2
33	Naringenin	30.68	MRM	Negative	270.95	151.2-119.3
34	Apigenin	31.43	MRM	Negative	268.95	117.3-151.2
35	Hesperetin	31.76	MRM	Negative	300.95	164.2-136.2
36	Kaempferol	31.88	MRM	Negative	284.75	255.1-117.3
37	Chrysin	36.65	MRM	Negative	252.95	143.3-119.4

3. RESULTS AND DISCUSSION

3.1. Optimization of Chromatographic Conditions

For the accurate identification of the analysed compounds, the HPLC–MS/MS analysis was achieved with electrospray ionization (ESI) mode using multiple reaction monitoring (MRM) which monitors the transitions of the parent to daughter ions of all standards. Analytes were characterized by their MS/MS spectra and retention time. For optimum MS results, ionization was accomplished in negative ESI mode and the precursor ions were corresponding to the deprotonated $[M-H^+]^-$ adducts. Quantification of target compounds was achieved after optimizing the acquisition parameters (Table **1**).

3.2. Method Validation

We validated the developed LC-MS/MS method according to linearity, precision, recovery study, limits of detection (LODs), limits of quantification (LOQs) and specificity (Table **2**). The method exhibited a good linearity of all standards ($R^2 \geq 0.990$) over a wide scale of concentrations (Table **2**). The method showed a good precision as the relative standard deviations (RSDs %) of the inter and intraday studies ranged from 0.058 to 3.209 % and 0.076 to 2.605 %; respectively. The extraction recoveries of the analyzed standards in the spiking study in the inter and intraday studies were found to be within the acceptable range (Table **2**). The percentage of recoveries ranged from 98.47 to 104.09 %. Thus, the matrix effect of the extracts was negligible for the assay. This method was sensitive as LODs and LOQs ranged from 0.003 to 0.821 and 0.004 to 0.859 µg/ml; respectively (Table **2**). The relative standard uncertainties were equal or less than 2.82% for all the analyzed compounds which means that the unknown true value is located at a maximum of ± 2.82% around the calculated result.

3.3. Application of HPLC–MS/MS Method to the Fractions of *O. fistulosa*

LC-MS/MS is the most reliable technique for determination of the phytochemical composition of plant extracts due to its high selectivity and sensitivity. So, the developed, optimized and validated LC-MS/MS method was applied for the simultaneous determination of 37 phytochemicals (Fig. **1A** & Table **1**) in the three fractions of *O. fistulosa* including 14 phenolic acids (gallic acid, protocatechic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, salicylic acid, *tr*-ferulic acid, sinapic acid, rosmarinic acid, cinnamic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid and *α*-coumaric acid), 3 non-phenolic organic acids (quinic acid, malic acid, fumaric acid), 17 flavonoids (rutin, hesperidin, isoquercitrin, nicotiflorin, rhoifoline, quercitrin, apigetrin, myricetin, fisetin, liquiritigenin, quercetin, luteolin, naringenin, apigenin, hesperetin, kaempferol and chrysin), a phenolic aldehyde (vanillin), a benzopyrone (coumarin) and a catechol (pyrocatechol). The results of LC-MS/MS analysis (Table **3** and Fig. **1**) showed that the analyzed fractions were rich in phenolic acids and flavonoids. The number of total phenolic compounds detected in dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BuOH) fractions were found to be 9, 15 and 12; respectively. This revealed that EA and BuOH fractions were richer in phenolic compounds than DCM fraction.

LC-MS/MS analyses revealed that the number of phenolic acids detected in the studied fractions was higher than the number of flavonoids (Table **3**). More specifically, 9 phenolic acids (gallic acid, protocatechic acid, chlorogenic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, salicylic acid, *p*-coumaric acid and *tr*-ferulic acid) were detected and among them chlorogenic acid (10052.5 µg/g), *tr*-ferulic acid (700.15 µg/g) and *p*-coumaric acid (309.62 µg/g) were the most abundant. While only 8 flavonoids (rutin, hesperidin, isoquercitrin, nicotiflorin, quercitrin, apigetrin, naringenin and apigenin) were detected and among them, apigetrin (10804.33 µg/g), rutin (2606.46 µg/g), and quercitrin (475.5 µg/g) were the most abundant. In addition, the 3 non-phenolic organic acids (quinic acid (267978.1µg/g), malic acid (2520.28µg/g) and fumaric acid (435.1µg/g)) were detected in large quantities. The highest amount of quinic and malic acids were detected in EA fraction (267978.1 and 2520.28 µg/g extract) followed by BuOH (95065.37 and 473.61 µg/g extract) and DCM (24784.31 and 285.15 µg/g extract) fractions. Whereas, chlorogenic acid was detected in large quantities in the BuOH (10052.5 µg/g extract) and EA (4731.3 µg/g extract) fractions and in a smaller amount in the DCM fraction (95.75 µg/g extract). Vanillic and *tr*-ferulic acids were detected in moderate amounts in DCM and EA fractions, while gallic, protocatechic, caffeic and salicylic acids were detected in EA and BuOH fractions. Fumaric acid was observed only in BuOH fraction and vanillin only in DCM fraction (165.21 µg/g extract).

The highest content flavonoid, apigetrin (Apigenin 7-O-glucoside) was detected in EA and BuOH fractions with values of 10804.33 and 8804.27 µg/g extract; respectively, while the lowest content flavonoid, nicotiflorin (a trihydroxyflavone linked to disaccharide) was detected only in BuOH fraction (15.07 µg/g extract). The aglycon naringenin (flavanone) was detected only in the DCM fraction (23.97 µg/g extract), while quercitrin (a tetrahydroxyflavone linked to monosaccharide) was found only in the EA fraction (475.47 µg/g extract). It is important to mention that the tetrahydroxyflavone, rutin (a disaccharide derivative) was observed in significant amounts only in the BuOH and EA fractions with values of 2606.46 and 554.60 µg/g extract; respectively. Isoquercitrin and hesperidin were detected only in EA and BuOH fractions with values of (151.95 and 70.81 µg/g extract) and (67.90 and 64.31 µg/g extract); respectively. The EA and DCM fractions contained moderate amounts of apigenin of 331.07 and 38.76 µg/g extract; respectively. Generally, flavonoids linked mainly to two monosaccharides were detected in the EA and BuOH fractions, more polar compounds than those detected in the DCM fraction (a flavone glucoside, a flavone, and a flavanone)

The identified phenolic compounds in the tested fractions of *O. fistulosa* were reported to have a beneficial effect on health and can also be exploited for phytopharmaceutical applications because of their biological properties [17, 18].

Table 2. Concentration range, linearity (R^2), Limits of Detection (LODs), Limits of Quantification (LOQs) and percentages of recoveries of the analysed 37 compounds by LC–MS/MS.

No.	Compounds	Conc. Range (Linearity Range) (µg/ml)	R2	LOD (µg/ml)	LOQ (µg/ml)	Inter-day (n=3) RSD (%)	Intra-day (n=3) RSD (%)	Recovery % (n = 3)		U (%)
								Inter-day	Intra-day	
1	Quinic acid	0.250-10	0.996	0.075	0.079	0.259	0.274	100.28	98.77	0.0082
2	Malic acid	0.250-10	0.999	0.055	0.067	0.477	0.527	101.26	99.83	0.0113
3	Fumaric acid	0.10-5	0.997	0.028	0.034	0.536	0.460	99.74	99.86	0.0124
4	Gallic acid	0.250-10	0.998	0.095	0.106	1.601	01.443	100.00	100.45	0.0282
5	Protocatechic acid	0.100-5	0.995	0.028	0.031	1.236	1.296	99.40	101.07	0.0411
6	Pyrocatechol	1-20	0.996	0.261	0.278	1.313	1.339	99.98	99.93	0.0235
7	Chlorogenic acid	0.025-1	0.998	0.006	0.008	0.058	0.076	100.80	99.96	0.0069
8	*p*-Hydroxybenzoic acid	0.250-10	0.998	0.033	0.038	1.284	1.538	99.66	100.05	0.0289
9	Vanillic acid	0.1-20	0.999	0.122	0.139	0.528	0.619	100.09	104.09	0.0508
10	Caffeic acid	0.025-1	0.998	0.018	0.022	1.454	1.469	100.91	98.82	0.0354
11	Syringic acid	0.1-20	0.996	0.021	0.233	1.049	1.345	99.92	99.97	0.0238
12	Vanillin	0.250-10	0.998	0.044	0.053	0.696	0.793	99.67	99.61	0.0280
13	Salicylic acid	0.025-1	0.989	0.005	0.006	1.016	1.242	100.98	99.01	0.0329
14	p-Coumaric acid	0.025-1	0.992	0.007	0.009	1.820	1.727	100.61	101.22	0.0516
15	Rutin	0.025-1	0.997	0.005	0.006	0.473	0.624	100.99	98.01	0.0159
16	tr-Ferulic acid	0.250-10	0.997	0.036	0.042	0.708	0.619	99.98	100.28	0.0494
17	Sinapic acid	0.250-10	0.992	0.078	0.086	1.446	1.517	100.16	99.96	0.0281
18	Hesperidin	0.025-1	0.998	0.003	0.004	0.945	1.126	101.73	101.26	0.0262
19	Isoquercitrin	0.025-1	0.999	0.005	0.006	0.682	0.515	100.59	100.72	0.0133
20	Rosmarinic acid	0.100-5	0.994	0.006	0.008	2.014	1.751	99.20	103.43	0.0713
21	Nicotiflorin	0.100-5	0.991	0.022	0.025	0.737	0.875	102.55	100.97	0.0276
22	α-Coumaric acid	0.025-1	0.999	0.024	0.031	2.730	2.566	98.34	99.06	0.0513
23	Rhoifolin	0.100-5	0.999	0.023	0.027	0.747	1.528	101.04	101.73	0.0941
24	Quercitrin	0.100-5	0.999	0.022	0.025	1.528	2.320	99.72	100.62	2.0079
25	Apigetrin	0.025-1	0.993	0.005	0.006	1.797	1.607	101.39	100.41	0.0597
26	Coumarin	1-20	0.994	0.208	0.228	1.306	1.239	99.94	100.08	0.0237
27	Myricetin	0.250-10	0.999	0.053	0.057	0.652	0.711	99.98	100.04	0.0126
28	Fisetin	0.250-10	0.991	0.054	0.051	0.557	0.820	99.87	100.03	0.0148
29	Cinnamic acid	5-20	0.996	0.821	0.859	0.648	0.816	100.05	99.92	0.0143
30	Liquiritigenin	0.025-1	0.996	0.005	0.006	1.849	1.738	100.33	99.95	0.0341
31	Quercetin	0.100-5	0.990	0.023	0.028	1.589	1.360	98.47	100.10	0.0543
32	Luteolin	0.025-1	0.997	0.005	0.006	0.575	0.696	100.77	99.52	0.0174
33	Naringenin	0.025-1	0.995	0.005	0.006	2.054	2.019	99.88	101.00	0.0521
34	Apigenin	0.025-1	0.990	0.005	0.006	2.304	2.204	101.44	101.33	0.0650
35	Hesperetin	0.025-1	0.997	0.005	0.006	3.209	2.605	98.85	99.43	0.0562
36	Kaempferol	1-20	0.992	0.206	0.214	1.436	1.070	99.97	99.85	0.0209
37	Chrysin	0.025-1	0.993	0.005	0.006	0.490	0.630	100.33	100.43	2.0083

RSD %: relative standard deviation.
U (%): uncertainty Percent at 95% confidence level (k = 2).

Table 3. Quantitative determination of 37 phenolic compounds in the extracts of *O. fistulosa* (µg/g extract) by LC-MS/MS, relative standard deviations (RSDs %) were in a range from 0.90 to 3.15%.

No.	Compounds	DCM Fraction	EA Fraction	BuOH Fraction
1	Quinic acid	24,784.31	267,978.1	95,065.37
2	Malic acid	285.15	2,520.28	473.61
3	Fumaric acid	N.I.	N.I.	435.10
4	Gallic acid	N.I.	67.85	62.29
5	Protocatechuic acid	N.I.	102.67	25.40
6	Chlorogenic acid	95.75	4731.30	10,052.5
7	*p*-Hydroxybenzoic acid	28	212.42	26.41
8	Vanillic acid	257.45	257.96	N.I.
9	Caffeic acid	N.I.	92.24	29.02
10	Vanillin	165.21	N.I.	N.I.
11	Salicylic acid	N.I.	7.22	2.22
12	*p*-Coumaric acid	26.64	309.62	28.37
13	Rutin	N.I.	554.60	2,606.46
14	*tr*-Ferulic acid	700.15	532.67	N.I.
15	Hesperidin	N.I.	67.90	64.31
16	Isoquercitrin	N.I.	151.95	70.81
17	Nicotiflorin	N.I.	N.I.	15.07
18	Quercitrin	N.I.	475.47	N.I.
19	Apigetrin	224.25	10,804.33	8,804.27
20	Naringenin	23.97	N.I.	N.I.
21	Apigenin	38.76	331.07	N.I.
	Total no. of detected compounds	11	17	15

DCM, Dichloromethane; EA, Ethyl acetate; BuOH, n-butanol; N.I., Not Identified.
The omitted metabolites were not detected.

3.4. Biological Activities

3.4.1. Antioxidant Activity, Total Phenolic and Flavonoid Contents

The investigations of medicinal plants as a potential source of natural antioxidants are necessary because they do not induce side effects like synthetic antioxidants [19]. Also, the plant extracts usually show chemical complexity, often a mixture of compounds with different polarities and chemical nature, which could lead to scattered and different results, according to the type of the assay. Therefore, assessment of the antioxidant potential of plant extracts with several tests would be more informative and even necessary [9, 20].

The results obtained (Table **4**) revealed that the three fractions of *O. fistulosa* are rich in phenolic and flavonoid compounds. The EA fraction showed the highest values (205.57 ± 3.93 mg GAE/g extract and 98.24 ± 0.04 mg QE/g extract) of phenolic and flavonoid contents respectively followed by DCM (200.21 ± 2.78 mg GAE/g extract and 97.84 ± 0.2 mg QE/g extract) and BuOH (175 ± 5.21 mg GAE/g extract and 42.04 ± 0.42 mg QE/g extract) fractions. More generally, the solubility of phenolic compounds depends on their chemical nature in the plant, which varies from simple to highly polymerized compounds such as phenolic acids, phenylpropanoids, anthocyanins, and tannins. This structural diversity is responsible for the wide variability of physico-chemical properties influencing the extraction of polyphenols [21].

3.4.1.1. β-carotene Bleaching Test

The IC_{50} results of β-carotene bleaching method (Table **4**) showed that the DCM fraction (0.77 ± 0.99 µg/ml) was the most active one followed by BuOH (3.44 ± 1.53 µg/ml) and EA (3.70 ± 1.88 µg/ml) fractions. Only DCM fraction was more potent as lipid peroxidation inhibitor than the tested standards (BHT, 1.34 ± 0.04 µg/ml; quercetin, 1.81 ± 0.11 µg/ml; and α-tocopherol, 2.10 ± 0.08 µg/ml).

3.4.1.2. DPPH Free Radical Scavenging Test

The IC_{50} values of DPPH scavenging test (Table **4**) revealed that the DCM fraction (6.66 ± 0.03 µg/ml) was the

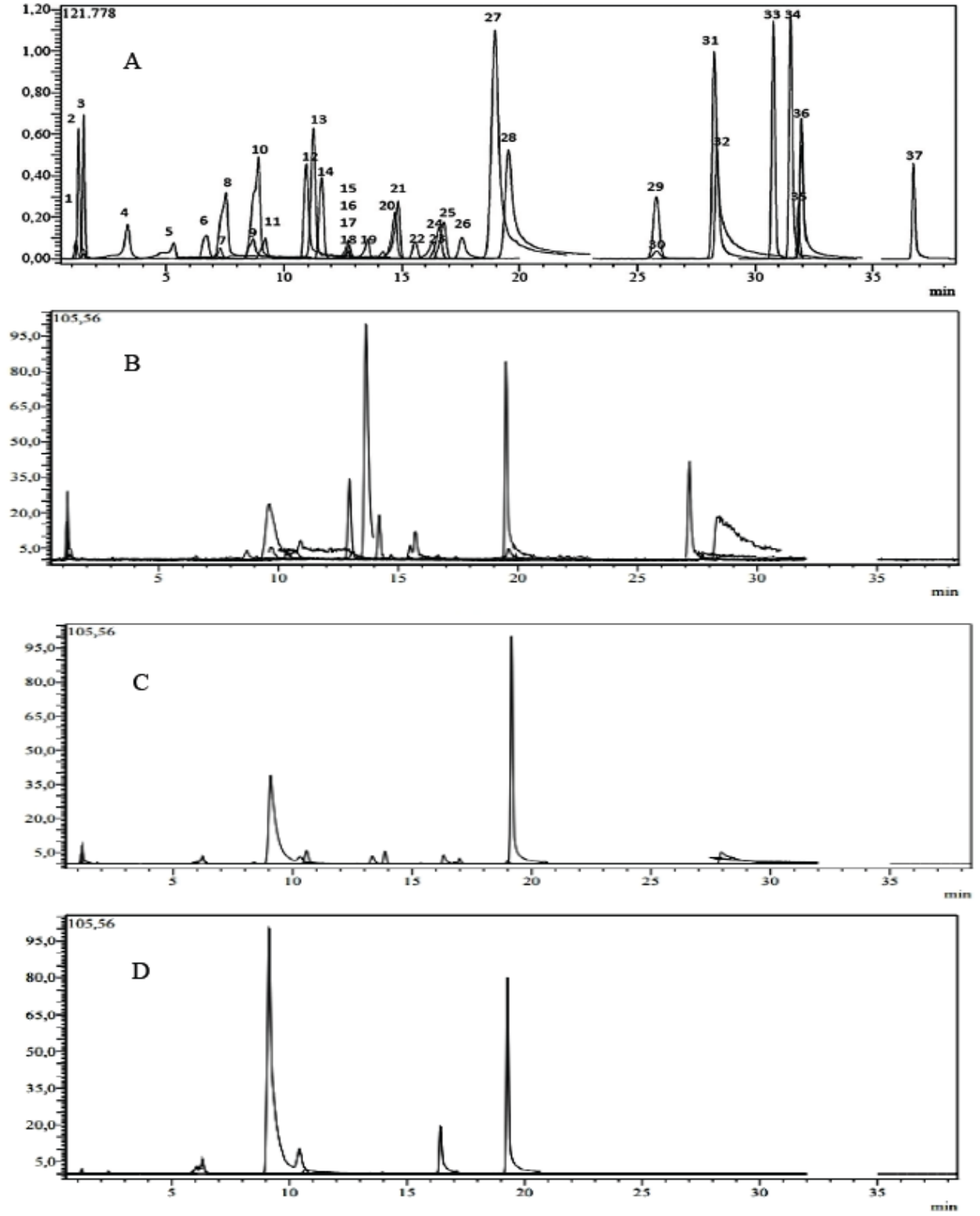

Fig. (1). LC-MS/MS chromatograms: (A) TIC chromatogram of the standards mixture (1µg/ml); (B) Chromatogram of dichloromethane (DCM) fraction of *O. fistulosa*; (C) Chromatogram of ethyl acetate (EA) fraction of *O. fistulosa*. (D) Chromatogram of n-butanol (BuOH) fraction of *O. fistulosa*. Legend**:** (1) quinic acid, (2) malic acid, (3) fumaric acid, (4) gallic acid,(5) protocatechic acid, (6) pyrocatechol, (7) chlorogenic acid, (8) 4-OH-benzoic acid, (9) vanillic acid, (10) caffeic acid, (11) syringic acid, (12) vanillin, (13) salicylic acid, (14)*p*-coumaric acid, (15) rutin, (16)*tr*-ferulic acid, (17) sinapic acid, (18) hesperidin, (19) isoquercitrin, (20) rosmarinic acid, (21) nicotiflorin, (22)*α*-coumaric acid, (23) rhoifolin, (24) quercitrin, (25) apigetrin, (26) coumarin, (27) myricetin, (28) fisetin, (29) cinnamic acid, (30) liquiritigenin, (31) quercetin, (32) luteolin, (33) naringenin, (34) apigenin, (35) hesperetin, (36) kaempferol and (37) chrysin.

Table 4. Total phenolic and flavonoid contents; and antioxidant activity of the fractions of *O. fistulosa* by the *β*-carotene-linoleic acid, $DPPH^{\cdot}$, $ABTS^{\cdot+}$, Phosphomolybdenum and CUPRAC assays.

Samples	Total Phenols[b]	Total Flavonoids[c]	*β*-carotene IC_{50} (µg/ml)	$DPPH^{\cdot}$ IC_{50} (µg/ml)	$ABTS^{\cdot+}$ IC_{50} (µg/ml)	Phospho-molybdenum IC_{50} (µg/ml)	CUPRAC $A_{0.50}$ (µg/ml)
DCM fraction	200.21 ± 2.78	97.84 ± 0.20	0.77± 0.99	6.66 ± 0.03	0.78 ± 0.51	276.83 ±1.61	20.35 ± 0.93
EA fraction	205.57 ± 3.93	98.24 ± 0.04	3.70 ± 1.88	16.09 ± 1.99	4.82 ± 0.43	184.33 ± 1.23	1.72 ± 0.11
BuOH fraction	175 ± 5.21	42.04 ± 0.42	3.44 ± 1.53	120.51± 1.23	11.88 ± 0.17	223.83 ± 0.97	3.31 ± 0.71
α-Tocopherol[a]	-	-	2.10 ± 0.08	7.31 ± 0.17	4.31± 0.16	-	10.20 ± 0.86
BHT[a]	-	-	1.34 ± 0.04	45.4 ± 0.47	4.10 ± 0.27	-	3.80 ± 0.33
Quercetin[a]	-	-	1.80 ± 0.11	2.07 ± 0.10	1.18 ± 0.03	-	-
Ascorbic acid[a]	-	-	-	-	-	793.64 ± 2.80	-
EDTA[a]	-	-	-	-	-	-	-

[a]Standard compounds, [b] mg gallic acid equivalent/g extract; [c] mg quercitin equivalent/g extract.
DCM, Dichloromethane; EA, Ethyl acetate; BuOH, n-butanol; N.I., Not Identified.

Table 5. Acetylcholinesterase, butyrylcholinesterase and tyrosinase inhibitory activities of the fractions of *O. fistulosa.*

Samples	AChE IC_{50} (µg/ml)	BChE IC_{50} (µg/ml)	Tyrosinase IC_{50} (µg/ml)
DCM fraction	9.94	139.43	184.70
EA fraction	10.11	6.54	1404.42
BuOH fraction	0.01	0.10	32.10
Galantamine[a]	50.90	7.39	-
Kojic acid[a]	-	-	0.67
L-Mimosine [a]	-	-	0.64

[a] Standard compounds
DCM, Dichloromethane; EA, Ethyl acetate; BuOH, n-butanol; N.I., Not Identified.

most active followed by EA fraction (16.09 ± 1.99 µg/ml) and BuOH fraction (120.51 ± 1.23 µg/ml). DCM fraction was more active than *α*-tocopherol (7.31 ± 0.17 µg/ml) and BHT (45.4 ± 0.47 µg/ml) and less active than quercetin (2.07 ± 0.10 µg/ml). Furthermore, EA fraction showed greater activity than BHT standard but, the BuOH fraction was less active than all the tested standards.

3.4.1.3. ABTS Radical-cation Reduction Test

According to the IC_{50} results (Table **4**) of the $ABTS^{\cdot+}$ trapping test, the DCM fraction (0.78 ± 0.51 µg/ml) showed the best activity, which was superior to that of quercetin (1.18 ± 0.03 µg/ml), BHT (4.10 ± 0.27 µg/ml) and *α*-tocopherol (4.31± 0.16 µg/ml). EA (4.82 ± 0.43 µg/ml) and BuOH (11.88 ± 0.17 µg/ml) fractions were less active than standards.

3.4.1.4. Total Antioxidant Activity Test

Phosphomolybdenum test is used primarily to measure the possibility and potency of non-enzymatic antioxidants. The results (Table **4**) indicated that DCM (276.83 ± 1.61 µg/ml), EA (184.33 ± 1.23 µg/ml) and BuOH (223.83 ± 0.97 µg/ml) fractions possessed better activity in reduction of Mo (VI) to Mo (V) than the standard ascorbic acid (793.64 ± 2.80 µg/ml). This activity may be due to the high content of phenolic compounds in the various fractions studied.

3.4.1.5. Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Test

According to the $A_{0.50}$ values of CUPRAC test (Table **4**), both EA (1.72 ± 0.11 µg/ml) and BuOH (3.31 ± 0.71 µg/ml) fractions demonstrated higher activity compared with those of the standards BHT (3.80 ± 0.33 µg/ml) and *α*-tocopherol (10.20 ± 0.86 µg/ml). While DCM fraction (20.35 ± 0.93 µg/ml) showed a weaker activity. In general, it can be concluded that all the fractions, especially the EA and BuOH fractions, exhibited very good and interesting antioxidant activity by the copper reduction method. Prior *et al.* [22] classified the CUPRAC antioxidant test as one of the methods based on electron transfer and advocated the superiority of this method over other antioxidant tests.

3.4.2. Anti-cholinesterase Activity Test

Herbs are viewed as an important natural source of new and safe cholinesterase inhibitor drugs, which could be used for the treatment of neurodegenerative disorders [23]. Compounds that exhibit anticholinesterase activity are also related to anti-radical or antioxidant activity [24]. In order to verify these approaches, anti-cholinesterase activity was evaluated for the fractions of the studied plant using galantamine as a standard compound. The anti-cholinesterase assay was performed against two enzymes, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). IC_{50} results of AChE inhibitory activity (Table **5**) revealed that all three fractions (DCM, EA, and BuOH) possessed very strong inhibitory activity (galantamine, 50.9 µg/ml) being BuOH fraction (0.01 µg/ml) the most active one followed by DCM (9.94 µg/ml) and EA (10.11 ± 2.68 µg/ml) fractions. The IC_{50} results of BChE showed that the BuOH (0.10 µg/ml) and EA (6.54 µg/ml) fractions possessed strong and better inhibitory activity than galantamine (7.39 µg/ml), being the DCM fraction the least active (139.43 µg/ml). So, tested polar fractions of *O. fistulosa* revealed a competitive acetylcholinesterase and butyrylcholinesterase inhibitory activities with that of galantamine.

3.4.3. Tyrosinase Inhibitory Activity Test

Tyrosinase is a metalloprotein that catalyzes the first two stages of melanogenesis and thus appears to be the limiting enzyme [25]. Melanin is responsible for pigmentation of skin and hair, but its production in excess amounts may lead to hyperpigmentation or vitiligo disease [26]. Due to the adverse effects of synthetic tyrosinase inhibitors currently being used, the look for new inhibitors of natural origin is necessary. So, we tested the tyrosinase inhibitory activity for the different fractions of *O. fistulosa* (Table **5**). Only the BuOH fraction (32.10 µg/ml) showed good activity, while DCM (184.70 µg/ml) and EA (1404.42 µg/ml) fractions were inactive in comparison to kojic acid (0.67 µg/ml) and L-mimosine (0.64 µg/ml) standards.

It has been accounted for that tyrosinase enzyme can be inhibited by aromatic aldehydes and acids, flavonoids and copper chelators [27]. This may explain the tyrosinase inhibitory activity of the BuOH fraction because it is rich in phenolic acids (*e.g.* chlorogenic acid) and flavonoids (especially rutin) according to our LC-MS/MS analysis. Furthermore, rutin was reported to be a potent antipigment agent due to its tyrosinase inhibitory activity [26]. Chlorogenic acid metabolic products can decrease melanogenesis in B16 melanoma cells by tryosinase inhibition [28]. The absence of tyrosinase inhibitory activity in DCM and EA fractions, could be explained by the absence of certain flavonoids or the presence of other components. Our results demonstrated that *O. fistulosa* (BuOH fraction) might be a candidate for hyperpigmentation disorders.

CONCLUSION

The current study was carried out in order to evaluate the chemical composition, especially of phenolic compounds in dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BuOH) fractions of *Oenanthe fistulosa* by LC-MS/MS. The number of phenolic compounds detected in DCM, EA and BuOH fractions were found to be 9, 15 and 12, respectively. The number of phenolic acids detected was higher than the number of flavonoids. More specifically, 9 phenolic acids were detected and among them, chlorogenic, *tr*-ferulic and *p*-coumaric acids were the most abundant ones. While only 8 flavonoids were detected, among them, apigetrin, rutin and quercitrin were the most abundant ones. In addition, 3 non-phenolic organic acids (quinic, malic and fumaric acids) were detected in large quantities. We determined the total phenolic and flavonoid contents, and EA fraction showed the highest values, followed by DCM and BuOH fractions. Furthermore, the antioxidant action was dictated by five methods and the tested fractions demonstrated a noteworthy antioxidant action. The study reports that the antioxidant effect of different fractions of *O. fistulosa* from our plant may be due to a synergism between polyphenols and other components. In addition, the tested fractions displayed a good inhibitory activity of the AChE and BChE enzymes; being the BuOH fraction the most potent one. Therefore, *O. fistulosa* may be an important source of potential new cholinesterase inhibitor drugs which could be utilized for the treatment of neurodegenerative disorders such as Alzheimer's disease. Finally, tyrosinase inhibitory activity was investigated but only BuOH fraction showed good activity. Therefore, *O. fistulosa* might be a promising candidate for hyperpigmentation disorders and we recommend it for potential applications in medicine and cosmetics as whitening agents.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors are grateful to the Departments of Chemistry at the University of Mugla (Turkey) and the University of Constantine 1 (Algeria) for providing funds to perform the present research.

REFERENCES

[1] Leurquin, J. Etude du genre Oenanthe (Apiaceae) de la Belgique et des régions voisines, Clés de détermination, Données morphologiques, stationne/les et socio- écologiques. *Lotissement Coputienne, 10- 6920 Wellin Janvier.,* **2007**, 26.

[2] Appendino, G.; Pollastro, F.; Verotta, L.; Ballero, M.; Romano, A.; Wyrembek, P.; Szczuraszek, K.; Mozrzymas, J.W.; Taglialatela-Scafati, O.; Taglialatela-Scafati, O. Polyacetylenes from sardinian *Oenanthe fistulosa*: a molecular clue to risus sardonicus. *J. Nat. Prod.,* **2009**, *72*(5), 962-965. [http://dx.doi.org/10.1021/np8007717] [PMID: 19245244]

[3] Stroh, P.A. Oenanthe fistulosa L. *Tubular Water Dropwort. Species Account. Botanical Society of Britain and Ireland,* https://bsbi.org/wp-content/uploads/dlm_ uploads/Oenanthe_ fistulosa_species_account.pdf **2015**.

[4] Lu, C.L.; Li, X.F. A Review of *Oenanthe javanica* (Blume) DC. as Traditional Medicinal Plant and Its Therapeutic Potential. *Evid. Based Complement. Alternat. Med.,* **2019**, *2019*6495819 [http://dx.doi.org/10.1155/2019/6495819] [PMID: 31057651]

[5] Valente, J.; Zuzarte, M.; Gonçalves, M.J.; Lopes, M.C.; Cavaleiro, C.; Salgueiro, L.; Cruz, M.T. Antifungal, antioxidant and anti-inflammatory activities of *Oenanthe crocata* L. essential oil. *Food Chem. Toxicol.,* **2013**, *62*, 349-354. [http://dx.doi.org/10.1016/j.fct.2013.08.083] [PMID: 24012643]

[6] Savo, V.; Salomone, F.; Bartoli, F.; Caneva, G. When the Local Cuisine Still Incorporates Wild Food Plants: The Unknown Traditions of the Monti Picentini Regional Park (Southern Italy). *Econ. Bot.,* **2019**, •••, 1-19. [http://dx.doi.org/10.1007/s12231-018-9432-4]

[7] Yilmaz, M.A.; Ertas, A.; Yener, I.; Akdeniz, M.; Cakir, O.; Altun, M.; Demirtas, I.; Boga, M.; Temel, H. A comprehensive LC-MS/MS method validation for the quantitative investigation of 37 fingerprint phytochemicals in Achillea species: A detailed examination of *A. coarctata* and *A. monocephala. J. Pharm. Biomed. Anal.,* **2018**, *154*, 413-424. [http://dx.doi.org/10.1016/j.jpba.2018.02.059] [PMID: 29602084]

[8] Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. Antioxidant activity of some algerian medicinal plants fractions containing phenolic compounds. *Food Chem.,* **2006**, *97*, 654-660. [http://dx.doi.org/10.1016/j.foodchem.2005.04.028]

[9] Öztürk, M.; Duru, M.E.; Kivrak, S.; Mercan-Doğan, N.; Türkoglu, A.; Özler, M.A. *In vitro* antioxidant, anticholinesterase and antimicrobial activity studies on three Agaricus species with fatty acid compositions and iron contents: a comparative study on the three most edible mushrooms. *Food Chem. Toxicol.,* **2011**, *49*(6), 1353-1360. [http://dx.doi.org/10.1016/j.fct.2011.03.019] [PMID: 21419821]

[10] Marco, G.J. A rapid method for evaluation of antioxidants. *J. Am. Oil Chem. Soc.,* **1968**, *45*, 594-598. [http://dx.doi.org/10.1007/BF02668958]

[11] Blois, M.S. Antioxidant determinations by the use of a stable free radical. *Nature,* **1958**, *181*, 1199-1200. [http://dx.doi.org/10.1038/1811199a0]

[12] Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.,* **1999**, *26*(9-10), 1231-1237. [http://dx.doi.org/10.1016/S0891-5849(98)00315-3] [PMID: 10381194]

[13] Ramalakshmi, K.; Kubra, I.R.; Rao, L.J.M. Antioxidant potential of low-grade coffee beans. *Food Res. Int.,* **2008**, *41*, 96-103. [http://dx.doi.org/10.1016/j.foodres.2007.10.003]

[14] Apak, R.; Güçlü, K.; Ozyürek, M.; Karademir, S.E. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J. Agric. Food Chem.,* **2004**, *52*(26), 7970-7981. [http://dx.doi.org/10.1021/jf048741x] [PMID: 15612784]

[15] Ellman, G.L.; Courtney, K.D.; Andres, V., Jr; Feather-Stone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.,* **1961**, *7*, 88-95. [http://dx.doi.org/10.1016/0006-2952(61)90145-9] [PMID: 13726518]

[16] Khatib, S.; Nerya, O.; Musa, R.; Shmuel, M.; Tamir, S.; Vaya, J. Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol moiety. *Bioorg. Med. Chem.,* **2005**, *13*(2), 433-441. [http://dx.doi.org/10.1016/j.bmc.2004.10.010] [PMID: 15598564]

[17] Heleno, S.A.; Martins, A.; Queiroz, M.J.R.; Ferreira, I.C. Bioactivity of phenolic acids: metabolites versus parent compounds: a review. *Food Chem.,* **2015**, *173*, 501-513. [http://dx.doi.org/10.1016/j.foodchem.2014.10.057] [PMID: 25466052]

[18] Tripoli, E.; La Guardia, M.; Giammanco, S.; Di Majo, D.; Giammanco, M. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chem.,* **2007**, *104*, 466-479. [http://dx.doi.org/10.1016/j.foodchem.2006.11.054]

[19] Falowo, A.B.; Fayemi, P.O.; Muchenje, V. Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. *Food Res. Int.,* **2014**, *64*, 171-181. [http://dx.doi.org/10.1016/j.foodres.2014.06.022] [PMID: 30011637]

[20] Sochor, J.; Zitka, O.; Skutkova, H.; Pavlik, D.; Babula, P.; Krska, B.; Horna, A.; Adam, V.; Provaznik, I.; Kizek, R. Content of phenolic compounds and antioxidant capacity in fruits of apricot genotypes. *Molecules,* **2010**, *15*(9), 6285-6305. [http://dx.doi.org/10.3390/molecules15096285] [PMID: 20877223]

[21] Koffi, E.; Sea, T.; Dodehe, Y.; Soro, S. Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. *J. Anim. Plant Sci.,* **2010**, *5*, 550-558.

[22] Prior, R.L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.,* **2005**, *53*(10), 4290-4302. [http://dx.doi.org/10.1021/jf0502698] [PMID: 15884874]

[23] Mustafa, A.M.; Eldahmy, S.I.; Caprioli, G.; Bramucci, M.; Quassinti, L.; Lupidi, G.; Beghelli, D.; Vittori, S.; Maggi, F. Chemical composition and biological activities of the essential oil from *pulicaria undulata* (L.) C. A. Mey. Growing wild in Egypt. *Nat. Prod. Res.,* **2018**, •••, 1-5. [http://dx.doi.org/10.1080/14786419.2018.1534107] [PMID: 30394109]

[24] Papandreou, M.A.; Dimakopoulou, A.; Linardaki, Z.I.; Cordopatis, P.; Klimis-Zacas, D.; Margarity, M.; Lamari, F.N. Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. *Behav. Brain Res.,* **2009**, *198*(2), 352-358. [http://dx.doi.org/10.1016/j.bbr.2008.11.013] [PMID: 19056430]

[25] Seo, B.; Yun, J.; Lee, S.; Kim, M.; Hwang, K.; Kim, J.; Min, K.R.; Kim, Y.; Moon, D. Barbarin as a new tyrosinase inhibitor from Barbarea orthocerus. *Planta Med.,* **1999**, *65*(8), 683-686. [http://dx.doi.org/10.1055/s-1999-14092] [PMID: 10630104]

[26] Si, Y.X.; Yin, S.J.; Oh, S.; Wang, Z.J.; Ye, S.; Yan, L.; Yang, J.M.; Park, Y.D.; Lee, J.; Qian, G.Y. An integrated study of tyrosinase inhibition by rutin: progress using a computational simulation. *J. Biomol. Struct. Dyn.,* **2012**, *29*(5), 999-1012. [http://dx.doi.org/10.1080/073911012010525028] [PMID: 22292957]

[27] Xie, L.P.; Chen, Q.X.; Huang, H.; Wang, H.Z.; Zhang, R.Q. Inhibitory effects of some flavonoids on the activity of mushroom tyrosinase. *Biochemistry (Mosc.),* **2003**, *68*(4), 487-491. [http://dx.doi.org/10.1023/A:1023620501702] [PMID: 12765534]

[28] Li, H.R.; Habasi, M.; Xie, L.Z.; Aisa, H.A. Effect of chlorogenic acid on melanogenesis of B16 melanoma cells. *Molecules,* **2014**, *19*(9), 12940-12948. [http://dx.doi.org/10.3390/molecules190912940] [PMID: 25157464]