Antioxidant activity and bioactive compounds of extracts from the Algerian plant *Moltkia ciliata*

Soumaia Chihi¹,², Oumelkheir Rahim¹, Ali Douadi¹* and Rabab Zatout³

¹ Pollution & Waste Treatment Laboratory, Kasdi Merbah University, Ouargla 30000, Algeria
² VTRS Laboratory, El-Oued University, B.P. 789, El-Oued 39000, Algeria
³ Department of pharmacy, Faculty of medicine, Benyoucef Benkhedda Algerian University, Algiers 16000, Algeria

alidouadi@gmail.com

Abstract. In the present study, we reported the evaluation of antioxidant properties using electrochemical and spectrophotometric assays and determination of total bioactive compounds content of the ethyl acetate (EtOAc) and n-butanol (n-BuOH) extracts of *Moltkia ciliata* growing in Algeria. The results obtained showed that the EtOAc extract was rich in bioactive compounds, which showed the most effective antioxidant capacity in all tests compared to the n-BuOH extract. This was confirmed by the chromatographic analysis and HPLC of the extract of many compounds in different quantities. The difference in antioxidant activity can be explained by differences in the levels of polyphenols and flavonoids. The study concludes this plant is a rich source of phenols and flavonoids, and also showed good in-vitro antioxidant activity by all methods. Thus, the plant *M. ciliata* can be explored as a potential source of natural antioxidant.

Keywords. *Moltkia ciliata*, bioactive compounds, Antioxidant capacity, HPLC, TLC.

1. Introduction

It was recently found that many serious diseases were caused by the use of many synthetic drugs as antioxidants [1]. Several recent studies have proven that the richness of plants in secondary plant metabolites qualifies them to be a source of medicinal agents [2,3]. Phytochemicals have shown several antibacterial, fungal and viral activities [4], as well as their use in several fields such as food, cosmetics, and others [5]. Despite their low molecular weight, polyphenols are responsible for antioxidant properties such as antioxidant, antimicrobial, antiallergic, anti-inflammatory and anticancer [6,7]. Several previous studies reported that several compounds had the property of inhibiting the removal of free radicals, such as phenolic compounds, tannins, anthocyanins and flavonoids, through different mechanisms [8]. *Moltkia ciliata* is a desert plant that is little known in phytochemistry and there is no purely chemical study about it, a contribution from us to shed light on it and to be the first phytochemical study on its effectiveness, this work was devoted to the chemical, antioxidant study of organic extracts of *Moltkia ciliata*, collected using the mixture (methanol/water) applying different analytical methodologies, by answering the following questions: To what extent does this plant contain active substances, especially phenolic compounds? What is the effect of the extraction method on the nature of the extracts? What is the extent of the antioxidant effectiveness of organic extracts?
2. Materials and methods

2.1. Chemicals and reagents
All chemicals, solvents and reagents were analytical grades or purest quality purchased and all used as received.

2.2. Plant Material

*Moltkia ciliata* is a species of plants in the family Boraginaceae. This family consists of about 131 genera, most of which are annual, semi-annual or perennial herbs, sub-shrubs, and the plants of this family are widespread in tropical and subtropical regions [9]. *M. ciliata* is a small, long-stemmed shrub that is wrapped with hard bristles, no longer than 30 cm long [10]. This plant is a food sanctuary for some desert animals especially camel; it has several traditional uses in the medical field used for abdominal diseases, wound healing and treatment against scorpion stings *M. ciliata* has attracted the interest of many researchers, especially in the statistical studies of some characteristics of desert plants [11].

![Figure 1. Outside of the plant Moltkia ciliata](image)

2.3. The phytochemical study

2.3.1. Phytochemical Screening
We performed several preliminary phytochemical tests to determine the different active substances present in the aerial part of the plant [12-14].

2.3.2. Extraction and isolation

The aerial parts of the plant (100 g) were soaked in the mixture (MeOH/H₂O) (80/20, v/v) (99 °C; Biochem Chemopharma (Canada)) for 24 h, the process was repeated three times, the filtrate was collected, and concentrated at 35 °C and then dissolved in distilled water. The resulting solution was treated by a series of successive liquid-liquid extractions using solvents of increasing polarity, starting with petroleum ether, then chloroform, then ethyl acetate and finally with n-butanol, phases are dried Organic magnesium sulphate anhydrous (97%, Acros Organics), and then its concentration.

2.3.3. Chromatographic Separation

Thin layer chromatography (TLC) is a quick and simple analytical technique used for the purpose of separating and identifying metabolites based on adsorption phenomena. This technique provides an overview of the compounds present in the extract [15].

2.3.4. Phenolic identification

By high-performance liquid chromatography HPLC (Shimadzu, LC 20, Japan) [16], the mobile phases were: (A: acetic acid, B: acetonitrile (v/v)), at rate (0.8 ml/min) and the detection wavelength was 285nm. Phenolic compound standards quercetin, vanillin, gallic acid, caffeic acid, ascorbic acid, rutin and chlorogenic acid were dissolved in solvents extraction and used for identification of the phenolic
acids present in different extracts of *M. ciliata*. Using the peak area of the reference compounds the quantification of individual phenolic compounds in the extracts is performed and expressed in mg/g of the extract.

2.4. Determination of total bioactive compounds content

2.4.1. Total phenolic content

The folin-ciocalteu reagent (FCR; 98%; biochem chemopharma (Canada)) was used to determine the total concentrations of the phenolic content of the extracts by following the method described by (Singleton Rossi 1999) [13], where we use the gallic acid (C₇H₆O₃; 99%; Alfa Aesar (Germany)) as reference phenol. 1ml of extract solution add 0.5ml of dilute folin ciocalteu reagent 10 times, after 5 min add 2 ml of sodium carbonate solution (Na₂CO₃; 7.5%) the mixture completed the reaction for 30 min at room temperature. The absorbance was read at 765 nm using a UV-Vis spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). Using the standard compound curve, the total phenol content was calculated and expressed in mg of gallic acid equivalent per gram of dry weight of the extract [8,17].

2.4.2. Total flavonoids content

The total flavonoids content were determined using the method of (Ordonez et al 2006) [18], where we use the rutin (C₁₅H₁₀O₇; 97%; Alfa Aesar (Germany)) as reference flavonoid. A volume of 0.5 ml of 2% AlCl₃ methanol solution was added to 0.5 ml of extract solution. The mixture was incubated for 1 h at room temperature for yellow color appearance; the absorbance was measured at 420 nm. The total flavonoids content was calculated from the calibration curve, and the results were expressed as mg of routine equivalent per g dry weight of extract [19].

2.4.3. Total flavanol content

The total flavanols content were determined using the method of Kumaran and Karunakaran (2007) [20], where we use the quercetin (C₁₅H₁₀O₇; 97%; Alfa Aesar (Germany)) as reference flavanol. 2.0 ml of the sample was mixed with 2.0 ml of 2% AlCl₃ prepared in methanol and 3.0 ml of 50 g/l sodium acetate solution were added. The mixture was incubated at 20 °C for 2.5 h after which the absorption was read at 440 nm using spectrophotometer. The total flavanol content was calculated from the calibration curve, and the results were expressed as mg of quercetin equivalent per g dry weight of extract [21]. The total experiment was conducted in triplicate and the results were expressed as means ± SD (standard deviation).

2.5. Antioxidant activities

2.5.1. Free radical scavenging activity

2.5.1.1. Free 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical-scavenging ability

Free radical scavenging activity of extracts were measured using a stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (C₁₅H₁₁N₃O₁·; Sigma-Aldrich Chemicals (St Louis, MO, USA)) and was determined spectrophotometrically by the slightly modified method of Djeridane et al. 2010 [22]. This test is done by mixing 1.5 ml of 100 μM DPPH in methanol with 1.5 ml of different concentrations of a solution of extracts and standards in the same solvent. Incubate in the dark for 30 minutes at room temperature. The absorbance is read in a UV-Vis spectrophotometer at 517 nm. Using the following equation (1), we were calculated the ability of the test sample to quench the root of DPPH.

\[
DPPH \text{ scavenging activity} (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

The activity strength of the samples was expressed as the IC50 concentration, which is the amount of antioxidant needed to inhibit 50% of the initial amount of DPPH [23].

2.5.1.2. Free superoxide anion radical \((\mathcal{O}_2^-)\) radical-scavenging ability

By using the electrochemical method that depends on the techniques of measuring periodic voltmeters, the antioxidant capacity of the studied extracts was measured, using PG301 potentiostat (radiometer analytical SAS) connected to an electrochemical cell of three electrode [24,25]. The antioxidant capacity
is calculated by the current density of the anodic curve of a voltammogram [25,26]. The superoxide anion radical was generated by one electron reduction of the commercial molecular oxygen (O₂) dissolved in DMF (analytical grade, Sigma-Aldrich) containing 0.1 M TNBHFP at room temperature (28±1°C), were used quercetin as a standard in the calculation of antioxidant capacity of the studied samples. The ability of the test sample to quench superoxide anion radicals (O₂⁻) (% Inhibition of (O₂⁻)) was calculated using the equation (2) [27,28], and according to the researcher’s method Bourvellec et al. [29]

$$O_2^- \text{ radical scavenging activity} \% = \frac{i_0 - i_t}{i_0} \times 100 \quad (2)$$

Where $i_0$ and $i_t$ are the anodic peak current densities of the superoxide anion radical in the absence and in the presence of the extract.

### 2.5.2. The ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to the method of Benzie and Strain (1996) with slightly modification [30]. The stock solutions included 300 mM acetate buffer (3.1 g C,H₉,N₃,O₂ and 16 ml C,H₅O₂), pH 3.6, 10mM TPTZ (2, 4, 6-tripyridyl-s-triazine; C₃,N₆,H₆;98(%); Sigma-Aldrich Chemicals (St Louis, MO, USA)) solution in 40 mM HCl, and 20 Mm FeCl₃·6H₂O solution. The FRAP solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5ml FeCl₃·6H₂O. The ascorbic acid (C₆H₇O₆; 99%); Alfa Aesar (Germany)) used a standard compound. 0.3 ml of ascorbic acid solutions was mixed with 2.7 ml of the FRAP solution. The mixture was Leave in the dark for 30 min and gets a violet color. The absorbance was measured at 593 nm in a spectrophotometer. All determinations were carried out in triplicate. Using the standard curve relationship of ascorbic acid, we evaluated the antioxidant activity of organic extracts and the results were expressed as ascorbic acid equivalent (AAE) in milligrams per gram dry extract.

$$\text{FRAP value} = \frac{A_{593nm} \text{of test sample}(\mu g/ml) \times \text{FeCl}_3\text{concentration}(\mu mol/l)}{4 \text{min} A_{593nm} \text{of FeCl}_3(\text{at conc. 1000}(\mu g/ml))} \quad (3)$$

### 2.5.3. Total antioxidant capacity (Molybdate ion reduction)

The total antioxidant capacity of the extracts was evaluated by phosphomolybdenum method [32]. In this method, the antioxidant compounds convert Mo (VI) to Mo (V), it is green in color [33]. The ascorbic acid used a standard compound. The reagent solution was prepared by mixing (sulphuric acid 0.6 M, sodium phosphate 28 mM (Rathburn Chemical (Walkerburn, Peebleshire, UK)), ammonium molybdate 4 mM ((NH₄)₂MoO₄; 98%); BioChem (Quebec, Canada)). 0.3 ml of ascorbic acid solutions was mixed with 3 ml of the detergent solution, leave it for an hour in a water bath temperature of 95°C, we get the green color [34]. Using the standard curve relationship of ascorbic acid, we evaluated the antioxidant activity of organic extracts and the results were expressed as ascorbic acid equivalent (AAE) in milligrams per gram dry extract.

### 2.5.4. ABTS Radical Cation Decolorization Assay

The cation radical scavenging activities of the extracts were determined using ABTS, 2,2’-azino-bis-(3-ethylbenothiazoline-6-sulphonic acid) diammonium salt (C₁₅H₂₃N₃O₅S₂;98(%); Sigma-Aldrich Chemicals (St Louis, MO, USA)) (Pellegrini and al., 1999) [35]. This method is based on removing the greenish blue color of the citric radical in the presence of an antioxidant molecule at a wavelength of 734 nm [36]. For ABTS assay, the stock solutions included 7 mM ABTS + solution and 2.4 mM potassium persulphate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS + solution with 60 ml methanol to obtain an absorbance of (0.706 ± 0.001) units at 734 nm using the spectrophotometer. Fresh ABTS + solution was then prepared for each assay. Plant extracts/standard (BHT; butylated hydroxytoluene; 99%); Sigma-Aldrich Chemicals (St Louis, MO, USA)) (1 ml) of different concentration was allowed to react with 1 ml of the ABTS + solution freshly prepared solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer [34]. The ABTS scavenging capacity of the extracts was compared with that of standard and percentage inhibition calculated using the following formula [37].

$$\text{ABTS radical scavenging activity} (%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (4)$$
Abscontrol: The absorbance of ABTS radical + methanol. AbsSample: The absorbance of ABTS radical + extracts/standard.

2.6. Statistical Analysis
Experimental values are given as means ± standard deviation (SD) of three replicates for antioxidant activity. Statistical calculations were carried out by OriginPro Version 9.1 software (OriginLab Corporation). Values of p < 0.05 were regarded as significant.

3. Results and discussion
3.1. Phytochemical study
3.1.1. Preliminary phytochemical
The obtained results (Table 1) showed the richness of this plant in most of the natural compounds, previous studies of plants of this genus (*Moltkia*) have proven that they contain most of these effective compounds.

| Compounds       | Results                                      |
|-----------------|----------------------------------------------|
| Flavonoids      | (+++) Appearance of a red color characteristic aglycone flavones |
| Alkaloids       | (+++) appearance of turbidity                |
| Sterols         | color changes from purple to blue or green(++) |
| Volatile oils   | (+) A distinctive odor emission              |
| Saponosides     | (++) Appearance of foam shaken in for 15 min|
| Tannins         | (+++) Appearance of a blue-green color       |
| Coumarins       | (-) no precipitate                           |
| Saponosides     | (-) no precipitate                           |

(+++): important Presence, (++): average Presence, (+): weak, presence and (-): absence

3.1.2. Extraction yield
It is calculated by the following equation (5),

\[ R\% = \left( \frac{m_f}{m_i} \right) \times 100 \]  

Where: \( m_f \); The mass of the organic extract obtained and \( m_i \); The mass of the plant sample

Note the difference in extraction yield between organic extracts; the polarity of both the solvent and the component affects the separation [38]. The extracts AcOEt and n-BuOH are of the highest value, where the study was confined around them.

3.1.3. HPLC analysis
The constituents in the different extracts were analyses by HPLC. The contents of these compounds in different extracts were determined according to the calibration curves, where y was the peak area and x was the concentration of analyte (0–80 µg/ml). The quantitative results are summarized in Figure 2, so that represents: A.A: Ascorbic Acid; G.A: Gallic Acid; Ch.A: Chlorogenic Acid; C.A: Caffeic Acid; V: Vanillin; Q: Quercetin; R: Rutin. As shown, all compounds are present in the extracts with different quantities. Rutin, caffeic acid, quercetin and chlorogenic acid are present in significant quantities. Ethyl acetate extract is richer in compounds than others; this is due to the difference of these compounds in terms of polarity.
Figure 2. Constituents content analyses by HPLC

3.1.4. Chromatographic separation
Chemical constituents of the extracts were separated on aluminum-backed thin layer chromatography (TLC) plates (Merck, silica gel 60, F254). The solvent system were used: (methanol/chloroform/eau; 5/20/0.5), (methanol/chloroform; 1/3), (methanol/chloroform/n-butanol; 1/1/1). The separate chemical compounds were examined using ultraviolet rays 254 nm and 365 nm and chemical reagents, and it was possible to predict the possibility of the presence of the following compounds [39,40]: Flavon or flavonol containing OH at position $C_5$ and OH at position $C_4$ replaced or deleted purple, some flavonoids contain OH in position $C_5$ or chalcones containing OH in position $C_4$ and lack OH on the aromatic ring B (brown), isoflavones do not contain OH at position $C_5$ free radiant blue, flavonol containing OH at position $C_5$ with or no free OH at position $C_3$ Yellow. Where 9 compounds were identified in (n-BuOH) extract, 13 compounds in (AcOEt) extract.

3.2. Total bioactive compounds content
Figure 3 represent the analytical data for phenolics content, flavonoids and flavanol of the extracts of $M. ciliata$. In comparison, AcOEt extract showed the higher quantities compared to n-BuOH extract. The AcOEt extract contains a large number of polyphenolic compounds and flavonoids mainly due to the polarity of polyphenols determined [41,42]. These compounds can have some pharmacological effects as well as beneficial for human consumption [43,44].
3.3. Antioxidant activity

Various methods were used to measure the antioxidant capacity of the studied extracts and the results obtained are shown in Table 2.

Table 2. Antioxidant activity of M. ciliata extracts as determined by the DPPH, FRAP, TAC, superoxide anion (O$_2^-$), ABTS assays

| Extracts | IC$_{50}$DPPH (mg/ml) | FRAP Value (mg/g) | IC$_{50}$ superoxide anion (O$_2^-$) (mg/ml) | IC$_{50}$ABTS (mg/ml) | TAC Value (mg/g) |
|----------|---------------------|------------------|------------------------------------------|---------------------|-----------------|
| Standard | 0.28 ± 0.03         | -                | 0.23±0.01                                | 0.093±0.02          | -               |
| AcOEt    | 0.06 ± 0.03         | 292.29 ± 0.02    | 0.51±0.01                                | 0.09±0.01           | 91.03 ± 0.04    |
| n-BuOH   | 0.10 ± 0.01         | 256.68 ± 0.02    | 1.02±0.03                                | 0.23±0.01           | 85.61 ± 0.05    |

3.3.1. Free radical scavenging activities study

3.3.1.a. Free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging ability

The DPPH radical scavenging ability of plant extracts and ascorbic acid standard shown in Figure 4, we note that increasing the concentration leads to an increase in inhibition, it is clear from the results obtained that the AcOEt extract shows the highest DPPH radical scavenging activity (0.20±0.02) which was significantly closer to that of standard antioxidant. The radical scavenging ability of the extract is related to the presence of total phenolic content (TPC), because these compounds contribute significantly to the antioxidant activity and act as free radical scavengers due to their redox properties [45].

![Figure 4](image-url)  
Figure 4. The DPPH radical scavenging activity of extracts and the ascorbic acid

3.3.1.b. Free superoxide anion radical (O$_2^-$) radical-scavenging ability

The following procedure was followed for the measurement of the antioxidant capacity: It was obtained cyclic voltammograms of a solution of (DMF + 0.1M TNBHFP) recorded at a scan rate of 100 mV/s on GC at 28°C (A) saturated with commercial oxygen and are shown in Figure 5, and the same way this has been done cyclic voltammograms of extracts and the standard are shown in Figure 6, then we calculate the percentage of inhibition.
The current density of the anode peaks to the more positive values. This activity is comparable with that of BHT, the standard antioxidant used in Table 3.3.3. The ferric reducing antioxidant power (FRAP) assay

EtOAc extract gave the best effective compared to n-BuOH extract, and this is because the flavonoids and phenolic acids present in the extracts exhibit strong antioxidant activity which is manifested in their ability to compound with mineral atoms, especially iron and copper [47]. The antioxidant compound present in the samples forms a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm by UV-Spectrophotometer [48].

3.3.3. Total antioxidant capacity (Molybdate ion reduction assay)

The total antioxidant capacity of studied M. ciliata extracts was determined by molybdate ion reduction method. The high antioxidant activity of these plant extracts is due to their flavonoid and phenol content [49].

3.3.4. ABTS radical scavenging activity

Bleaching of ABTS cation has been extensively used to evaluate the antioxidant capacity [45]. The extracts from M. ciliata were fast and effective scavengers of ABTS$^+$ radical Figure 7 and the obtained results are shown in Table 2. This activity is comparable with that of BHT, the standard antioxidant used.
in this study. The percentage inhibition was 78.8%, 96.7% and 80.2% in n-butanol, ethyl acetate and BHT respectively at 0.5 mg/ml, the highest concentrations tested. Higher concentrations of the extracts were more effective in quenching free radicals in the system.

**Figure 7.** ABTS radical scavenging activities of extracts of *M. ciliata*

EtOAc extract gave the best effective compared to *n*-BuOH extract. It turns out that there is a relationship between ABTS$^+$ radical scavenging activity for extracts and Total phenolic, flavonoids content and confirms this compounds have contributes directly to the antioxidant effects of these extracts.

4. Conclusion

The ethyl acetate extract exhibited the highest activity in the all assays followed by *n*-butanol extract. This also proves that polyphenolic compounds and antioxidant activities are closely linked. The results obtained herein are in agreement to a certain degree with the traditional uses of *M. ciliata* as a valuable source for antioxidant drugs.

**Acknowledgements**

The authors would like to thank to Algerian Ministry of Higher Education and Scientific Research and General Directorate of Scientific Research and Technological Development (DGRSDT) for their support and providing the necessary facilities to carry out this research.

**References**

[1] Tzima K, Brunton N P and Rai D K 2018 *Plants* 7(2) 1.
[2] Klejduš B and Kováčik J 2016 *Industrial Crops and Products* 83 774.
[3] Leja K B and Czaczyk K 2016 *Acta Scientiarum Polonorum Technologia Alimentaria* 15(4) 353.
[4] Gopi Krishnan S, Ajith A P, Renish C, Duraiselvan S and Subash Maruthu Pandian K 2018 *Int. J. S. Res. Sci. Engg. Tech.* 4(8) 443.
[5] Engel R, Szabo K, Abranko L, Rendes K, Fuzy A and Takas T 2016 *Journal of agricultural and food chemistry* 64(19) 3733.
[6] Quideau S, Deffieux D, Douat-Casassus C and Pouyseg L 2011 *Angewandte Chemie International Edition* 50(3) 586.
[7] Oliveira A S, Ribeiro-Santos R, Ramos F, Castilho M C and Sanches-Silva A 2018 *Food analytical methods* 11(2) 440.
[8] Singh S and Vyas M 2016 *International Journal of Pharmaceutical Sciences and Research* 7(4) 1793.
[9] RaNjbaR M and Khalvat I S 2019 *Phytotaxa* 408(3) 143.
[10] Odeja O, Ene Ogwuche C, Emeka Elemike E and Obi G 2017 *Clinical Phytoscience* **2**(1) 1.
[11] Cohen J I 2014 *Cladistics* **30**(2) 139.
[12] Savithramma N, Rao M L and Ankanna S 2011 *International Journal of Research in Pharmaceutical Sciences* **2**(4) 643.
[13] Singleton V L, Orthofer R and Lamuela-Raventós R M 1999 *Methods in enzymology* **299** 152.
[14] Shihka R and Savaïta D 2017 *International Journal of Natural Products Research* **7**(2) 7.
[15] Hadij Salem J 2009 *Doct. Thesis* (France: Institut National Polytechnique de Lorraine) p 251
[16] Özen T 2010 *Grasas y aceites* **61**(1) 86.
[17] Kivrak Ş, Gökþürk T, Kivrak I, Kaya E and Karababa E 2019 *Food Science and Technology* **39**(2) 423.
[18] Ordonez A, Gomez J and Vattuone M 2006 *Food chemistry* **97**(3) 452.
[19] Phull A R, Abbas Q, Ali A, Raza H, Zia M and Haq I U 2016 *Future Journal of Pharmaceutical Sciences* **2**(1) 31.
[20] Kumaran A and Karunakaran R J 2007 *LWT Food Science and Technology* **40**(2) 344.
[21] Mbaebie B, Edeoga H and Afolayan A 2012 *Asian Pacific Journal of Tropical Biomedicine* **2**(2) 118.
[22] Djeridane A, Youssi M, Brunel J M and Stocker P 2010 *Food and Chemical Toxicology* **48**(10) 2599.
[23] Santos C M and Silva A 2020 *Molecules* **25**(3) 696.
[24] Ghiabi Z, Youssi M, Hadjadji M, Saidi M and Dakmouche M 2014 *International Journal of Electrochemical Science* **9**(2) 909.
[25] Anjomshoaa M, Fatemi S J, Torkzadeh-Mahani M and Hadadzadeh H 2014 *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **127** 511.
[26] Douadi K, Chaafia S, Douati T, Al-Noaïmi M and Kaabi I 2020 *Journal of Molecular Structure* **121** 7128305.
[27] Wu J, Bi S Y, Sun X Y, Zhao R, Wang J H and Zhou H F 2019 *Journal of Biomolecular Structure and Dynamics* **37**(13) 3496.
[28] Hui N, Niu X L, Han J Y, Sun W and Jiao K 2010 *Amino acids* **38**(3) 711.
[29] Le Bourvellec C, Hauchard D, Darchen A, Burgot J L and Abasq M L 2008 *Talanta* **75**(4) 1098.
[30] Benzie I F and Strain J J 1996 *Analytical biochemistry* **239**(1) 70.
[31] Franco D, Munekata P E, Agregán R, Bermúdez R, López-Pedrouso M, Pateiro M and Lorenzo J M 2020 *Antioxidants* **9**(2) 90.
[32] Celep E, Aydn A, Kirmizibekmez H and Yesilada E 2013 *Food and chemical toxicology* **62** 448.
[33] Rubio C P, Hernández-Ruiz J, Martínez-Subiela S, Tvarijonaviciute A and Cerón J J 2016 *BMC veterinary research* **12**(1) 99.
[34] Kamath S D, Arunkumar D, Avinash N G and Samshuddin S 2015 *Advances in Applied Science Research* **6**(6) 99.
[35] Re R, Pellegrini N, Proteggenta A, Pannala A, Yang M and Rice-Evans C 1999 *Free radical biology and medicine* **26**(9) 1231.
[36] Coulombier N, Nicolau E, Le Dénan L, Antheaume C, Jauffrais T and Lebouvier N 2020 *Marine drugs* **18**(2) 122.
[37] Youn J S, Kim Y J, Na H J, Jung H R, Song C K, Kang S Y and Kim J Y 2019 *Food science and biotechnology* **28**(1) 201.
[38] Kobus-Cisowska J, Szczepaniak O, Szymanska-Powalowska D, Piechocka J, Szulc P and Dziedziński M 2020 *Ciência Rural* **50**(1).
[39] Rahim O, Hamra H, Guerra H and Aliaoui M 2015 *Revue des Bioresources* **5**(1) 188.
[40] N‘gaman K C C, Bédro Y A, Mamyrbékova-Bédro J A, Bénié A and Gouré S 2009 *Eur. J. Sci. Res.* **36**(2) 161.
[41] Felhi S, Saoudi M, Daoud A, Hajlaoui H, Ncir M, Chaabane R, El-Feki A F, Gharsallah N and Kadri A 2017 *Food Science and Technology* **37**(4) 558.
[42] Tian S, Shi Y, Zhou X, Ge L and Upur H 2011 *Pharmacognosy magazine* **7**(25) 65.
[43] Bakari S, Hajlaoui H, Daoud A, Mighri H, Ross-García J M, Gharsallah N and Kadri A 2018 *Food Science and Technology* **38**(2) 310.
[44] Zeng L B, Zhang Z R, Luo Z H and Zhu J X 2011 *Food chemistry* **125**(2) 456.
[45] Tayag C M, Lin Y C, Li C C, Liou C H and Chen J C 2010 *Fish & shellfish immunology* 28(5-6) 764.
[46] Ahmed S and Shakeel F 2012 *Czech Journal of Food Sciences* 30(2) 153.
[47] Rabeta M and Faraniza R N 2013 *International Food Research Journal* 20(4) 1691.
[48] Phatak R S and Hendre A S 2014 *Journal of Pharmacognosy and Phytochemistry* 2(5) 32.
[49] Khiya Z, Oualcadi Y, Gamar A, Amalich S, Berrekhis F, Zair T and Hilali F E 2019 *Phytothérapie* 17(6) 321.