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### ► To cite this version:

Aicha Bouratoua, Assia Khalfallah, Chawki Bensouici, Zahia Kabouche, Abdulmagid Alabdul Magid, et al.. Chemical composition and antioxidant activity of aerial parts of *Ferula longipes* Coss. ex Bonnier and Maury. *Natural Product Research*, 2017, pp.1 - 8. 10.1080/14786419.2017.1353513 . hal-01834121

**HAL Id: hal-01834121**

**<https://hal.univ-reims.fr/hal-01834121v1>**

Submitted on 5 Nov 2021

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## Chemical composition and antioxidant activity of aerial partsof *Ferula longipes* Coss. ex Bonnier and Maury

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**KEYWORDS** *Ferula longipes* coss. ex Bonnier and Maury; apiaceae; flavonoid; antioxidant activity; reducing power assays

### ABSTRACT

This is the first study on the phytochemistry and antioxidant activity of *Ferula longipes* Coss. ex Bonnier and Maury (Apiaceae). A new flavonoid quercetin-3-*O*- $\alpha$ -L-rhamnopyranoside-7-*O*- $\beta$ -D-[2-*O*-caffeoyl]-glucopyranoside (**1**), along with 10 known compounds kaempferol-3-*O*- $\alpha$ -L-rhamnopyranoside (**2**), quercetin-3-*O*- $\alpha$ -L-rhamnopyranoside (**3**), kaempferol-3-*O*- $\beta$ -D-glucopyranoside-7-*O*- $\alpha$ -L-rhamnopyranoside (**4**), isorhamnetin-3-*O*- $\alpha$ -L-rhamnopyranoside-7-*O*- $\beta$ -D-glucopyranoside (**5**), quercetin-3-*O*- $\alpha$ -L-rhamnopyranoside-7-*O*- $\beta$ -D-glucopyranoside (**6**), isorhamnetin-3,7-di-*O*- $\beta$ -D-glucopyranoside (**7**), apigenin (**8**), apigenin-7-*O*- $\beta$ -D-glucopyranoside (**9**), 3,5-

dicafeoylquinic acid (**10**), deltoin (**11**) were isolated from the aerial parts of *Ferula longipes* Coss. Structures elucidation was performed by comprehensive 1D and 2D NMR analyses, mass spectrometry and by comparison with literature data. The compounds **1**, **3**, **4**, **6**, **7** and **10** were evaluated for their antioxidant activity, compound **1** exhibited the best antiradical activity potential and showed IC<sub>50</sub> and A<sub>0.5</sub> values 5.70, 7.25, 5.00, and 2.63  $\mu$ g/mL towards DPPH free radical-scavenging, ABTS, CUPRAC, and reducing power assays, respectively compared with BHA, BHT and ascorbic acid which were used as positive controls.

## 1. Introduction

The genus *Ferula* L. (Apiaceae) is represented by about 208 perennial herbaceous species occurring from Central Asia, westward throughout the Mediterranean region to Northern Africa and the Macaronesian Region (Mabberley 2008). Some species of this genus have been used throughout the history in traditional medicine, but also as spices and in human diet. Many *Ferula* species were used for upper respiratory conditions (asthma, bronchitis, etc.), for gastrointestinal disorders as spasmolytic, antifatulence, and antidiarrhoeal agent, as well as anthelmintic and aphrodisiac herbal drugs (Sadraei et al. 2001; Hadidi et al. 2003; Iranshahy & Iranshahi 2011). Previous chemical investigations of the genus *Ferula* have led to the identification of more than 200 compounds, including, sesquiterpene coumarins and coumarin esters (Ahmed 1999; Iranshahy & Iranshahi 2011; Meng et al. 2013; Razavi & Janani 2015; Asghari et al. 2016; Dastan et al. 2016; Razavi et al. 2016) and prenylated coumarins (Appendino et al. 1988; Arnoldi et al. 2004). Sesquiterpene coumarins, the most abundant class of secondary metabolites in *Ferula* species, are biologically active compounds and so far they have demonstrated promising chemopreventive, antitumour, anti-inflammatory and antileishmanial activities (Nazari and Iranshahi 2011). Plants of this genus also contain sesquiterpene chromone derivatives (Meng et al. 2013), daucane esters (Appendino et al. 1997), prenyl-benzoyl-furanone-type sesquiterpenoids (Kojima et al. 1999) and essential oils (Labeled-Zouad et al. 2015; Maggi et al. 2016). The present work describes the isolation and structural identification of a new flavonoid (**1**) and 10 known compounds (**2–11**) from the aerial parts of *Ferula longipes* Coss. In addition, the antioxidant activity of the compounds **1**, **3**, **4**, **6**, **7** and **10** was investigated.

## 2. Results and discussion

The *n*-BuOH and the ethyl acetate fractions of the aerial parts of *F. longipes* Coss. were fractionated and purified by combination of chromatographic methods to obtain a new flavonoid quercetin-3-*O*- $\alpha$ -L-rhamnopyranoside-7-*O*- $\beta$ -D-[2-*O*-caffeoyl]-glucopyranoside (**1**) and ten known compounds (**2–11**). They were elucidated as kaempferol-3-*O*- $\alpha$ -L-rhamnopyranoside (**2**) (Berrehal et al. 2010), quercetin-3-*O*- $\alpha$ -L-rhamnopyranoside (**3**)

(Berrehal et al. 2010), kaempferol-3-*O*- $\beta$ -D-glucopyranoside-7-*O*- $\alpha$ -L-rhamnopyranoside (**4**) (Berrehal et al. 2010), isorhamnetin-3-*O*- $\alpha$ -L-rhamnopyranoside-7-*O*- $\beta$ -D-glucopyranoside (**5**) (Wolbiś, 1989), quercetin-3-*O*- $\alpha$ -L-rhamnopyranoside-7-*O*- $\beta$ -D-glucopyranoside (**6**) (Maji and Banerji 2016), isorhamnetin-3,7-di-*O*- $\beta$ -D-glucopyranoside (**7**) (Yokozawa et al. 2002), apigenin (**8**) (Nacer et al. 2006), apigenin-7-*O*- $\beta$ -D-glucopyranoside (**9**) (Markham and Chari 1982), 3,5-dicaffeoylquinic acid (**10**) (Timmermann et al. 1983), deltoin (**11**) (Bensalem et al. 2013). Their structural assignments were made by ESI-MS, 1D-, and 2D-NMR analyses and by comparison with spectral data from the literature values. Compound **1** was isolated as a yellow amorphous powder. The positive ESI-MS spectrum showed the pseudo-molecular ion [M + Na]<sup>+</sup> at *m/z* 795.1758, compatible with the molecular formula of C<sub>36</sub>H<sub>36</sub>O<sub>19</sub>.

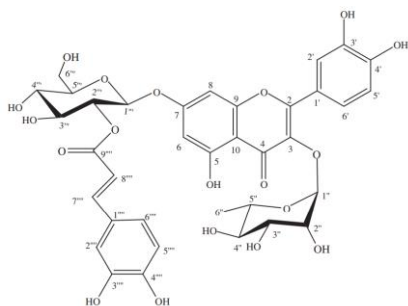
In the UV spectrum of compound **1** recorded in MeOH and MeOH + NaOH, a flavonol or a substituted flavonol at C3 with a free hydroxyl group at C-4' (Mabry et al. 1970; absorption bands were observed at 256, 346 nm and 266, 394 nm, respectively indicating Markham and Chari 1982).

The <sup>1</sup>H-NMR spectrum of compound **1** shows a typical pattern of quercetin aglycone. The two doublets at  $\delta$  6.42 and 6.71 (*J* = 2.2 Hz) assignable to H-6 and H-8, respectively were downfield indicating that there is a substitution at C7 (Berrehal et al. 2010) An ortho and meta coupled ABX-type protons were also observed at  $\delta$  6.92 (1H, d, *J* = 8.3 Hz, 5'-H), 7.33 (1H, dd, *J* = 8.3, 2.0 Hz, 6'-H), and 7.37 (1H, d, *J* = 2.0 Hz, 2'-H). In addition, 1H-NMR spectrum of **1** exhibited two doublets at  $\delta$  5.39 (1H, d, *J* = 1.5 Hz, H-1'') and at  $\delta$  5.37 (1H, d, *J* = 7.9 Hz, H-1''') assignable to two anomeric protons. The sugars were identified as  $\beta$ -glucopyranosyl (glc) and  $\alpha$ -rhamnopyranosyl (rham) on the basis of the 1H-1H COSY and HSQC spectra. The *J* values of their anomeric protons indicated the anomeric configurations of  $\beta$ -glc and  $\alpha$ -rham. On acid hydrolysis, compound **1** gave D-glucose and L-rhamnose which were identified by R<sub>f</sub> values on TLC with authentic samples (Touafek et al. 2011). Another ABX spin system can be assigned as  $\delta$  6.78 (1H, d, *J* =

8.2 Hz, 5<sup>'''</sup>-H), 6.96 (1H, dd,  $J = 8.2, 2.0$  Hz, 6<sup>'''</sup>-H), 7.06 (1H, d,  $J = 2.0$  Hz, 2<sup>'''</sup>-H). Furthermore, two doublets with a splitting of  $J_{7''',8'''} = 15.8$  Hz clearly indicated *trans* caffeoyl group.

In the HMBC spectrum a cross peak was observed between C-7 at  $\delta$  162.7 and 1<sup>'''</sup>-H at  $\delta$  5.37 indicating the attachment of the  $\beta$ -D-glucose at C-7 of quercetin. Another HMBC correlation was observed between C-3 at  $\delta$  135.1 and H-1<sup>''</sup> at  $\delta$  5.39 indicating the linkage position of the  $\alpha$ -L-rham. Along range correlation was observed between H-2<sup>'''</sup> ( $\delta$  5.13, 1H, dd,  $J = 9.6, 7.9$  Hz) and the caffeoyl-carbonyl carbon  $\delta$  166.8 indicating that caffeoyl group was attached to C-2<sup>'''</sup> of the glc. Consequently, the structure of compound **1** was determined as quercetin-3-*O*- $\alpha$ -L-rhamnopyranoside-7-*O*- $\beta$ -D-[2-*O*-caffeoyl]-glucopyranoside (Figure 1).

The antioxidant activity of compounds **1**, **3**, **4**, **6**, **7** and **10** was tested using DPPH free radical-scavenging, CUPRAC, ABTS and reducing power assays. BHA, BHT and ascorbic acid were used as positive standards. The tests were performed at different concentrations to calculate the IC<sub>50</sub> and A<sub>0.50</sub> values. Results were statistically significant ( $p < 0.05$ ) when compared with those of controls in each test



**Figure 1.** chemical structure of compound **1** isolated from *Ferula longipes* coss. ex Bonnier and Maury.

In DPPH and ABTS assays, all compounds have a high-scavenging activity, compound **1** showed the best antiradical activity (IC<sub>50</sub>: 5.70  $\pm$  0.82 and 5.00  $\pm$  0.65  $\mu$ g/mL, respectively) followed by compound **3** (IC<sub>50</sub>: 7.37  $\pm$  0.31 and 5.94  $\pm$  0.08  $\mu$ g/mL, respectively) and **6** (7.53  $\pm$  0.34 and 6.07  $\pm$  0.37  $\mu$ g/mL, respectively) with a higher antiradical activity than the standards BHA (IC<sub>50</sub>: 7.96  $\pm$  1.01 and 5.56  $\pm$  0.77  $\mu$ g/mL, respectively) and BHT (IC<sub>50</sub>: 14.73

$\pm$  0.52 and 8.52  $\pm$  0.67  $\mu$ g/mL, respectively). Compounds **7** (IC<sub>50</sub>: 9.42  $\pm$  0.59 and 6.13  $\pm$  0.72  $\mu$ g/mL, respectively), **10** (IC<sub>50</sub>: 12.87  $\pm$  0.09 and 10.12  $\pm$  1.01  $\mu$ g/mL, respectively) and **4** (IC<sub>50</sub>: 15.57  $\pm$  0.32 and 11.54  $\pm$  0.09  $\mu$ g/mL, respectively) also showed a good activity compared with the standards.

In the same order, with the CUPRAC and reducing power assays, compounds **1** (A<sub>0.50</sub>: 7.25  $\pm$  0.12 and 2.63  $\pm$  0.55  $\mu$ g/mL, respectively), **3** (A<sub>0.50</sub>: 7.85  $\pm$  0.32 and 2.75  $\pm$  0.24  $\mu$ g/mL, respectively) and **6** (A<sub>0.50</sub>: 8.50  $\pm$  0.40 and 2.86  $\pm$  0.14  $\mu$ g/mL, respectively) exhibited a higher activity than the standards BHA (A<sub>0.50</sub>: 8.56  $\pm$  0.36 and 3.23  $\pm$  0.27  $\mu$ g/mL, respectively), BHT (A<sub>0.50</sub>: 8.75  $\pm$  0.11 and 3.83  $\pm$  0.67  $\mu$ g/mL, respectively) and ascorbic acid (A<sub>0.50</sub>: 8.01  $\pm$  1.46  $\mu$ g/mL). Compounds **7** (A<sub>0.50</sub>: 9.23  $\pm$  0.72 and 3.02  $\pm$  0.82  $\mu$ g/mL, respectively), **10** (A<sub>0.50</sub>: 11.56  $\pm$  0.89 and 15.08  $\pm$  0.77, respectively) and **4** (A<sub>0.50</sub>: 13.48  $\pm$  0.38 and 23.01  $\pm$  0.50  $\mu$ g/mL, respectively) were also found to be active. In all antioxidant assays, the flavonoids **1**, **3** and **6** showed a higher antioxidant activity than the standards. Compound **4** had only one -OH group but **1**, **3** and **6** have two *ortho* -OH groups which increases the antioxidant activity (Muhammad et al. 2016). The antioxidant activity of **7** is higher than that of **4** because the H at C-3' position has been replaced by OCH<sub>3</sub> group in the B ring.

### 3. Experimental

#### 3.1. General experimental procedures

<sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in CD<sub>3</sub>OD on a Bruker AVANCE DRX 600 NMR spectrometer (Karlsruhe, Germany) (<sup>1</sup>H at 600 MHz and <sup>13</sup>C at 150 MHz). 2D-NMR experiments were performed using standard Bruker sequences. ESI-MS experiments were performed using a Micromass Q-TOF instrument (Manchester, UK).

Polyamide SC6 was purchased from Fluka (Steinheim, Germany). Silica gel 60 F<sub>254</sub> pre-coated aluminium plates (0.2 mm, Merck) were used for VLC analysis. The VLC spots were visualised under UV light (254 and 366 nm) followed by spraying with 50% H<sub>2</sub>SO<sub>4</sub> and heating. Column chromatography was carried out on Kieselgel 60 (63–200 mesh) Merck.

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulphonic Butylated-hydroxyl anisole (BHA), butylatedhydroxytoluene (BHT), ascorbic acid, acid) diammonium salt (ABTS), neocuproine, tri-chloro acetic acid, ferric chloride, potassium ferricyanide, were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). Electronic Single channel micropipette (Eppendorf AG, Hamburg, Germany). All other chemicals and solvents were of analytical grade.

### Plant material

Aerial parts of *F. longipes* Coss. ex Bonnier and Maury were collected from Bejaia (North-east of Algeria) in May 2012 and authenticated by Prof. Gérard De Belair (University of Annaba, Algeria). The voucher specimen (LOST/FI/05/12) has been kept in the Herbarium of the Faculty of Sciences (University of Mentouri Constantine).

### 3.2. Extraction and isolation

Air-dried and powdered aerial parts (900 g) of *F. longipes* Coss. were macerated in a methanolic solution (70%). The residue was filtered, concentrated and successively extracted with petroleum ether, dichloromethane, ethyl acetate and *n*-butanol.

The *n*-butanol fraction (14 g) was concentrated under reduced pressure and subjected to a column chromatography on polyamide SC6 with a gradient of toluene–MeOH of increasing polarity to give three fractions [F<sub>1</sub>: 300 mg, F<sub>2</sub>: 550 mg, F<sub>3</sub>: 900 mg]. Fraction F<sub>1</sub> was sub-

jected to a column chromatography on silica gel eluted with the system AcOEt–MeOH–H<sub>2</sub>O (10:1:0.5) affording four compounds, **1** (14 mg), **2** (28 mg), **3** (11 mg), and **8** (20 mg). Compound **4** (12 mg) precipitated in fraction F<sub>2</sub>. Fraction F<sub>3</sub> was subjected to a silica gel CC using the isocratic system *n*-hexane / acetone (9/1), to obtain compounds **5** (39 mg) and **6** (26 mg).

The ethyl acetate fraction (6 g) was concentrated under reduced pressure and subjected to fractionation by liquid chromatography under vacuum (VLC) over C18 reverse phase, eluted with the system H<sub>2</sub>O

/ MeOH (60:40, 40:60, 30:70, 20:80, 0:100) to obtain 7 fractions. Fraction F<sub>3</sub> (870 mg) was chromatographed on a silica gel CC with the isocratic system CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O (10:1:0.5), to obtain compounds **7** (25 mg) and **10** (31 mg). Fraction F<sub>5</sub> (405 mg) was chromatographed on a silica gel column with the isocratic system AcOEt:MeOH:H<sub>2</sub>O (8:2:1) leading to compounds **9** (25 mg) and **11** (16 mg).

#### 3.2.1. Quercetin-3-O- $\alpha$ -L-rhamnopyranoside-7-O-[2-O-caffeoyl]- $\beta$ -D-glucopyranoside (1)

Yellow amorphous powder;  $[\alpha]_D^{20} +41.2$  (c 0.20, MeOH), mp 329 °C, UV  $\lambda_{max}$  (MeOH, nm):256, 346 and (MeOH + NaOH, nm): 266, 394, <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) :  $\delta_H$  6.42 (d, *J* = 2.2 Hz, H-6), 6.71 (d, *J* = 2.2 Hz, H-8), 7.37 (d, *J* = 2.0 Hz, H-2'), 6.92 (d, *J* = 8.3 Hz, H-5'), 7.33 (dd, *J* = 8.3, 2.0 Hz, H-6'), 5.39 (d, *J* = 1.5 Hz, H-1''), 4.23 (dd, *J* = 3.3, 1.7 Hz, H-2''), 3.75 (dd, *J* = 9.5, 2.9 Hz, H-3''), 3.34 (t, *J* = 9.5 Hz, H-4''), 3.42 (m, H-5''), 0.94 (d, *J* = 6.1 Hz, H-6''), 5.37 (d, *J* = 7.9 Hz, H-1'''), 5.13 (dd, *J* = 9.6, 7.9 Hz, H- 2'''), 3.76 (t, *J* = 9.5 Hz H- 3'''), 3.54 (t, *J* = 9.4 Hz, H- 4'''), 3.65 (m, H- 5'''), 3.99 (dd, *J* = 12.2, 2.2 Hz, H-6'''a), 3.78 (dd, *J* = 12.2, 5.8 Hz, H-6'''b), 7.06 (d, *J* = 2.0 Hz, H-2''''), 6.78 (d, *J* = 8.2 Hz, H-5''''), 6.96 (dd, *J* = 8.2, 2.0 Hz, H-6''''), 7.63 (d, *J* = 15.8 Hz, H-7''''), 6.33 (d, *J* = 15.8 Hz, H- 8''''), <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD):  $\delta_C$  156.6 (C-2), 135.1 (C-3), 178.4 (C-4), 161.6 (C-5), 99.2 (C-6), 162.8 (C-7), 94.5 (C-8), 158.5 (C-9), 106.4 (C-10), 126.3 (C-1'), 115.6 (C-2'), 145.0 (C-3'), 148.6 (C-4'), 121.3 (C-5'), 121.6 (C-6'), 102.1 (C-1''), 70.5 (C-2''), 69.9 (C-3''), 71.8 (C-4''), 61.0 (C-5''), 16.2 (C-6''), 98.3 (C-1'''), 74.6 (C-2'''), 71.8 (C-3'''), 70.6 (C-4'''), 77.2 (C-5'''), 60.9 (C-6'''), 73.3 (C-1''''), 113.7 (C- 2''''), 146.2 (C-3''''), 148.3 (C-4''''), 115.1 (C- 5''''), 121.6 (C-6''''), 145.4 (C-7''''), 113.3 (C- 8''''), 166.8 (C-9''''). HR-ESI-MS *m/z* 795.1758 [M + Na]<sup>+</sup> [calc for C<sub>36</sub>H<sub>36</sub>O<sub>19</sub>Na, 795.1749].

#### 3.2.2. Acid hydrolysis

The pure compound was treated with 2 M HCl at 100 °C for 1 h. The hydrolysate was extracted with EtOAc. The two sugars were identified as

glucose and rhamnose in the aqueous residue by comparison with authentic samples on silica gel TLC impregnated with 0.2 M NaH<sub>2</sub>PO<sub>4</sub> [solvent Me<sub>2</sub>CO–H<sub>2</sub>O (9:1)] and revealed with aniline malonate. The optical rotation of each purified sugar was measured and compared with authentic samples to afford D-glucose and L-rhamnose.

### 3.3. Antioxidant activity

Four methods were used to evaluate the antioxidant activity: DPPH free radical-scavenging, ABTS, CUPRAC and reducing power assay.

#### 3.3.1. DPPH free radical-scavenging assay

The free radical-scavenging activity of compounds **1**, **3**, **4**, **6**, **7** and **10** isolated from *F. longipes* Coss. was determined by the DPPH assay (Blois 1958; Lakhali et al. 2011). In its radical form, DPPH absorbs at 517 nm, a 0.1 mM solution of DPPH in methanol was prepared and 160 μL of this solution was added to 40 μL of sample solutions in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. The following equation was used to calculate the scavenging of DPPH radical: % inhibition  $[(A_{b_{control}} - A_{b_{sample}}) / A_{b_{control}}] \times 100$ . DPPH solution in MeOH was used as a control. The curve of the % scavenging activity against the concentration of sample was prepared by the MS Excel-based programme to obtain the IC<sub>50</sub>. All the tests were conducted in triplicate. The experimental data were expressed as mean ± standard deviation.

#### 3.3.2. ABTS cation radical decolourisation assay

The ABTS scavenging activity was done by the method of Re et al. (1999) with slight modifications. The ABTS was produced by the reaction between 7 mM ABTS in H<sub>2</sub>O and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. The oxidation of ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 h had elapsed. The radical cation was stable in this form for more than 2 days with storage in the dark at room temperature. Before usage, the ABTS solution was diluted to get an absorbance of 0.700 ± 0.020 at 734 nm with ethanol. Then, 180 μL of ABTS solution

were added to 20 μL of sample solution in methanol at different concentrations. After 10 min, the percentage inhibition at 734 nm was calculated for each concentration relative to a blank absorbance (methanol). The scavenging capability of ABTS was calculated using the following equation: ABTS<sup>•+</sup> scavenging activity

#### Cupric reducing antioxidant capacity (CUPRAC) assay

CUPRAC was determined according to the method developed by Apak et al. (2004). The method comprises mixing of 40 μL of antioxidant solution with 50 μL of a copper(II) chloride solution, 50 μL of neocuproine alcoholic solution, and 60 μL of ammonium acetate aqueous buffer at pH 7. After 30 min the absorbance was read at 450 nm.

#### 3.3.3. Reducing power assay

The reducing power of the tested compounds was determined according to the method of Oyaizu (1986). 10 μL of sample solutions at different amounts were mixed with 40 μL of 0.2M phosphate buffer (pH 6.6) and 50 μL of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min. After that 50 μL of TCA (10%) was added and mixed with distilled water (40 μL) and 10 μL of ferric chloride (0.1%), the absorbance was read at 700 nm. Higher absorbance of the reaction mixture indicates greater reducing power.

## 4. Conclusions

In summary, one new flavonoid, along with ten known compounds, were isolated from the aerial parts of *F. longipes* Coss. Compounds **1**, **3**, **4**, **6**, **7** and **10** exhibited a high antioxidant activity towards DPPH free radical scavenging, ABTS, CUPRAC and reducing power assays, respectively compared with BHA, BHT and ascorbic acid which were used as positive controls.

#### Acknowledgements

The authors are grateful to Ministry of DGRSDT-MESRS (Algeria) and to and the Ministry of Higher Education and Research and to CRBt (Constantine-Algeria).

#### Disclosure statement

No potential conflict of interest was reported

by the authors.

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