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# Evaluation of antidiabetic, dermatoprotective, neuroprotective and antioxidant activities of *Chrysanthemum fontanesii* flowers and leaves extracts

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ABSTRACT

This study was aimed to evaluate the antidiabetic, dermatoprotective, neuroprotective and antioxidant activities of *n*-butanol extracts from leaves and flowers of *Chrysanthemum fontanesii*. β-carotene-linoleic acid, Galvinoxyl radical (GOR), ABTS, hydroxyl radical, reducing power, CUPRAC, O-phenanthroline and metal chelating assays were used to evaluate antioxidant capacity of the extracts. The extracts showed good antioxidant activity and were a good source of phenolics and flavonoids. The enzyme inhibitory activity of the extracts was investigated against key enzymes involved in neurodegenerative [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE], type 2 diabetes (α-glucosidase) and skin (tyrosinase) disorders. In the cholinesterase inhibitory assays, the extracts of leaves and flowers exhibited weak activity against AChE and BChE. *n*-butanol extracts from leaves and flowers displayed high inhibitory activity against α-glucosidase, showing potential properties against type 2 diabetes. Whereas for anti-tyrosinase activity, only flowers extract showed good inhibitory activity. Furthermore the leaves and flowers extracts showed high photoprotective activity with the sun protection factor (SPF) value: 38.96 ± 0.26 and 38.66 ± 0.68, respectively. Our study on *C. fontanesii* open new perspectives for developing novel health-promoting agents by pharmaceutical, cosmetics and food industries.

## 1. Introduction

The *Chrysanthemum* genus (Asteraceae) is very common in Mediterranean basin countries. This genus includes nearly 300 species, of which fifteen grow in Algeria. Aerial parts of *Chrysanthemum* are used as traditional drug, mainly for the treatment of constipation, intestinal transit problems and menstrual disorders in Tunisia. For instance, some *Chrysanthemum* species such as *C. trifurcatum* is consumed as food, especially, flowers (Ben Sassi et al., 2014).

Previous studies demonstrated that Algerian *C. fontanesii* possessed antioxidants, antibacterial, anti-inflammatory, hepatoprotective and nephroprotective effects (Amrani et al., 2016). *C. fontanesii* is almost completely uninvestigated from the chemical points of view, with only tow previous study reported in the literature that showed the presence of flavonoids, phenolic acid, sterol (Benaissa et al., 2011) and essential oil (Lograda et al., 2013).

Enzyme inhibition is a common and important method for discovery of new drugs and treatment of human public disorders. There are several enzymes whose inhibition are considered a target for the treatment or prevention of related diseases (Asghari et al., 2018), including cholinesterases (Alzheimer's disease), glucosidase and amylase (diabetes mellitus) and tyrosinase (Parkinson's disease, pigmentation).

Due to the increasing attention in the valorization of endemic plants to support their use in the pharmaceutical, cosmetic, and food industry, the *n*-butanol extracts of aerial parts from *C. fontaneisii* were studied herein. The potential applications of the *C. fontaneisii* were investigated in a wide multidisciplinary approach for different biological activities, such as antioxidant, photoprotective and enzyme inhibition.

*Abbreviations:* ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid; AChE, Acetylcholinesterase; BChE, Butrylcholinesterase; CUPRAC, cupric reducing antioxidant capacity; GOR, Galvinoxyl radical; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; OH, Hydroxyl radical; QE, quercetin equivalents; TPC, Total phenolic content; TFC, Total flavonoid content

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## 2. Materials and methods

### 2.1. Plant collection and identification

The aerial parts of *C. fontanesii* (leaves and flowers) were collected during the flowering season from Bejaia, Algeria and its scientific identification was performed by Prof. Mohamed Kaabache. The voucher specimen (CCF05/04/03) was deposited at Mentouri Brothers Constantine 1 University, Faculty of Nature and Life Sciences Herbarium.

### 2.2. Preparation of the extracts

The aerial parts of *C. fontanesii* (leaves: 1500 g and flowers: 1516 g) are cut into small pieces and macerated in an Methanol/water (80/20) mixture for 24–48 hours. This operation is repeated three times with solvent renewal. After concentration at a temperature not exceeding 35 °C, we obtained a syrupy residue. The latter is diluted with distilled water at a rate of 600 mL per 1 kg of dry matter and then added lead acetate [$(CH_3COO)_4$ Pb] to remove chlorophyll by precipitation. After filtration, the aqueous phase obtained is successively exhausted by liquid-liquid extraction in a separatory funnel using water-immiscible solvents of increasing polarity (chloroform, ethyl acetate and *n*-butanol). The organic phases thus obtained are dried with anhydrous sodium sulphate to remove all traces of water, then filtered and finally concentrated to dryness under reduced pressure and weighed.

### 2.3. Determination of total phenolic content (TPC)

TPC was determined using the modified Folin–Ciocalteu method of Singleton and Rossi, 1965. 20 µL sample (1 mg/mL) was mixed with 100 µL Folin–Ciocalteu reagent (diluted ten-fold) and 75 µL (75 g/L) sodium carbonate. Absorbance was measured at 740 nm in the microplate reader after 2 h incubation in darkness at room temperature. Gallic acid (25–500 µg/mL) was used as standard for calibration curve and construction of a linear regression line. TPC is expressed as µg gallic acid equivalent (GAE)/mg extract (Müller et al., 2010).

### 2.4. Determination of total flavonoid content (TFC)

TFC was determined using the modified method of Topçu et al., 2007. 50 µL extract (1 mg/mL) was added 10 µL of 10% aluminium nitrate, 10 µL of 1 M potassium acetate and 130 µL of methanol. The absorbance was read spectrophotometrically at 415 nm after 40 min incubation at room temperature, Quercetin was used as standard. TFC is expressed as quercetin equivalent (QE)/mg extract.

### 2.5. Flavonols content

The content of flavonols was determined by Kumaran and Karunakaran (2007) method. 50 µL of methanolic solution of plant extract with 50 µL (20 mg/mL) aluminium trichloride and 150 µL (50 mg/mL) sodium acetate. The absorbance was read spectrophotometrically 440 nm after 150 min. The content of flavonols was calculated using a calibration curve of standard quercetin.

### 2.6. Antioxidant activities

#### 2.6.1. Galvinoxyl free radicals (GOR) scavenging assay

160 µL of 0.1 mM methanolic solution of Galvinoxyl was added to 40 µL of different concentrations of extract (sample) in methanol. The absorbance was read at 428 nm after 120 min incubation in dark at room temperature. Galvinoxyl solution in methanol was used as a control (Shi et al., 2001).

The following equation was used to calculate the scavenging of galvinoxyl radical:

$$\text{Inhibition (\%)} = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100.$$

#### 2.6.2. ABTS cation radical decolourisation assay

$ABTS^{\bullet+}$ scavenging activity was determined spectrophotometrically according to the modified method of Re et al. (1999). Briefly, 160 µL of diluted $ABTS^{\bullet+}$ solution (7 mm ABTS in water and 2.45 mm potassium persulfate, stored in the dark at room temperature for 12 h, absorbance of 0.700 ± 0.020 at 734 nm) was added to 40 µL of extract (sample) in ethanol at different concentrations. After 10 min the absorbance was measured at 734 nm.

The above equation was used to calculate the scavenging of $ABTS^{\bullet+}$ radical. Where A Control is the initial concentration of the $ABTS^{\bullet+}$ and A Sample is the absorbance of $ABTS^{\bullet+}$ in the presence of sample.

#### 2.6.3. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by the modified method of (Smirnoff and Cumbes, 1989). Briefly, 40 µL extract was mixed with 80 µL salicylic acid (3 mM), 24 µL $FeSO_4$ (8 mM) and 20 µL $H_2O_2$ (20 mM). After incubation for 30 min at 37 °C, 36 µL $H_2O$ was added and the absorbance of the mixtures was measured at 510 nm. Ascorbic acid is used as the positive control (standard).

#### 2.6.4. O-phenanthroline assay

The reaction mixture consisted of 30 µL O-phenanthroline (0.5% in methanol), 50 µL $FeCl_3$ (0.2%), 110 µL Methanol, and 10 µL of various concentrations of the extract. The mixture was incubated for 20 min at 30 °C, then the absorbance of the same was measured at 510 nm and the percentage inhibition was calculated (Szydlowska-Czerniaka et al., 2008).

#### 2.6.5. Cupric ion reducing antioxidant capacity (CUPRAC)

Cupric ion reducing antioxidant capacity of the extracts was determined according to the modified method described by Apak et al. (2004). 50 µL $CuCl_2$ (10 mM), 50 µL neocuproine (7.5 mM), and 60 µL $NH_4Ac$ buffer (1 M, pH 7.0) solutions were added to each well, in a 96 well plate. 40 µL extracts at different concentrations was then added to the mixture (Total volume 200 µL) and mixed well. Absorbance against a reagent blank was measured at 450 nm after 1 h. Results were given as absorbances and compared with standards (BHT and BHA).

#### 2.6.6. Reducing power assay

10 µL of sample solutions at various concentrations, 40 µL of 0.2 M phosphate buffer (pH 6.6) and 50 µL of potassium ferricyanide (1%) were mixed and incubated at 50 °C in water bath for 20 min. After cooling, 50 µL of 10% trichloroacetic acid (TCA) were added, and the mixture was centrifuged for 10 min at 1000 rpm 40 µL of upper layer of solution was mixed with distilled water (40 µL) and 10 µL of ferric chloride (0.1%). The absorbance was read spectrophotometrically at 700 nm. Water is used as blank (Oyaizu, 1986).

#### 2.6.7. Metal chelating activity assay

Different concentrations of extracts or EDTA (40 µL) were added to 40 µL of $FeCl_2$ (0.2 mM) and 40 µL of methanol. The reaction was initiated by the addition of 80 µL of ferene solution (0.5 mM). The obtained samples were incubated for 10 min at room temperature after a vigorous stirring. The absorbance was read spectrophotometrically at 593 nm. The above equation was used to calculate the $Fe^{2+}$ chelating effect.

#### 2.6.8. β-carotene bleaching inhibition activity

Chloroform solutions (1 mL) of 0.5 mg of β-carotene, 200 mg of Tween 40 and 25 µL of linoleic acid were mixed and then the solvent was removed in vacuum, then 100 mL of distilled water saturated with oxygen were added to the mixture with vigorous stirring. To 160 µL of

residual emulsion was added 40 µL of samples (different concentrations). The mixtures were heated at 50 °C for 2 h and then the absorbances were measured at 470 nm (Marco, 1968) and the percentage of the antioxidant activity (AA) was calculated using the following formula:

AA% = (Abs of β-carotene content after 2 h /Abs of initial β-carotene content) × 100

### 2.7. Determination of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity

Different concentrations of extracts or galantamine (10 µL), 150 µL of sodium phosphate buffer (100 mM, pH 8.0), and 20 µL AChE ($5.32 \times 10^{-3}$ U) or BChE ($6.85 \times 10^{-3}$ U) solution were mixed and incubated for 15 min at 25 °C, and then 10 µL of 0.5 mM DTNB was added. The reaction was then initiated by the addition of 10 µL of acetylthiocholine iodide (0.71 mM) or 10 µL of butyrylthiocholine chloride (0.2 mM). The absorbance was read at 412 nm utilizing a 96-well microplate reader.

% of inhibition of AChE or BChE enzymes was determined using the formula (E-S)/E × 100, where E is the activity of enzyme without test sample, and S is the activity of enzyme with test sample (Ellman et al., 1961; Öztürk et al., 2011).

### 2.8. α-Glucosidase inhibition activity

50 µl of sample (extracts or acarbose) solution in100 mM sodium phosphate buffer (pH 6.9) and 50 µl of 5 mM *p*-Nitrophenyl α-D-Glucopyranoside solution (in phosphate buffer) was mixed and incubated for 5 min at 37 °C. 100 µl of 0.1 U/ml α-glucosidase from Baker's yeast (in phosphate buffer) was then added. Absorbance at 405 nm was recorded for 30 min using a microplate reader set to 37 C against a blank (Lordan et al., 2013). The activity of α-glucosidase was calculated as follows:

% *α-Glucosidase inhibition activity* = Absorbance of sample/Absorbance of control × 100

### 2.9. Tyrosinase inhibition activity

Tyrosinase inhibition enzyme activity was measured by the spectrophotometric method as described by Deveci et al. (2018). Briefly, different concentration of sample (extracts or kojic acid) solution dissolved in ethanol (10 µL), 150 µL of 100 mM sodium phosphate buffer (pH 6.8), and 20 µL tyrosinase enzyme (from mushroom) solution in buffer were mixed and incubated at 37 °C for 10 min, then 20 µL L-DOPA was added. After 10 min incubation at 37 °C the absorbances were read.

### 2.10. Photoprotective activity (in vitro sun protection factor determination)

The extracts was diluted in absolute methanol to obtain a concentration of 2 mg/mL. The aliquot prepared were scanned Spectrophotometrically between 290 and 320 nm, at 5 nm intervals, taking methanol as blank, using Shimadzu UV/VIS-Spectrophotometer. SPF value was calculated by the application to the equation developed by Mansur et al. (1986):

$$SPF_{spectrophotometric} = CF \times \sum_{290}^{320} EE(\lambda) \mathrm{x}\, I(\lambda) \mathrm{x}\, Abs(\lambda)$$

Where EE(λ) is the erythemal effect spectrum; I(λ) is the solar intensity spectrum; Abs(λ) is the absorbance; and CF is the correction factor (=10).

The values of EE(λ) × I(λ) are constants, and they were determined by Sayre et al. (1979).

### 2.11. Statistical analysis

The experiments were performed in triplicate. Results are given as a mean ± standard deviation (SD) of three parallel measurements. The data was analysed by one-way ANOVA (GraphPad Prism 5 software) followed by Turkey's post hoc test for multiple comparisons and $P < 0.05$ was considered as statistically significant.

## 3. Results

### 3.1. Total phenol content

The total phenol contents of *C. fontanesii* leaves and flowers extracts were quantified. The regression equation of calibration curve of gallic acid was y = 0.0034x + 0.1044 with $R^2$ = 0.9972. The results showed that the linear relationship was good in the detection ranges. *C. fontanesii* leaves had the highest total phenol content (883.99 ± 4.98 µg GAE/mg extract), followed by flowers (636.20 ± 9.88 µg GAE/mg extract).

### 3.2. Total flavonoids content

The content of flavonoids in plant extracts from leaves and flowers of *C. fontanesii* is determined using spectrophotometric method with $AlCl_3$ and expressed in terms of quercetin equivalent, QE (the standard curve equation: y = 0.0048x, $R^2$ = 0.997), µg QE/mg extract. The contents of flavonoids identified in the tested extracts are shown in Table 1. The highest content of flavonoids is measured in leaves (275.82 ± 2.20 µg QE/mg extract), while flowers extract contains small amount of flavonoids (166.80 ± 4.41 µg QE/mg extract) in the comparison to leaves extract.

### 3.3. Flavonols content

The flavonols content of *C. fontanesii* leaves and flowers is determined as quercetin equivalent (QE). The highest level of flavonol contents was measured in the leaves extract (118.47 ± 6.26 µg QE/mg extract) compared with flowers extract (70.77 ± 1.75 µg QE/mg extract).

### 3.4. Antioxidant properties

The antioxidant and free radical scavenging potentiel of the extracts is given in Tables 2 and 3. In GOR and ABTS assays, the extracts showed a concentration dependent increase in the scavenging activity. The *n*-butanol extract of leaves exhibited highest activity ($IC_{50}$ values: 8.49 ± 0.59 and 5.25 ± 0.21 µg/mL, respectively). It displayed superior free radical scavenging activity than that of flowers extract ($IC_{50}$ values: 15.35 ± 0.28, 10.32 ± 0.88 µg/mL, respectively), but lower

**Table 1**
Yield, total phenol content (TPC), total flavonoid content (TFC) and flavonol content.

| *Samples* | Yield % (g/g DW) | TPC (µg GAE/mg extract) | TFC (µg QE/mg extract) | Flavonol content (µg QE/mg extract) |
|---|---|---|---|---|
| *n*-Butanol extract of Flowers | 3.96 | 636.20 ± 9.88 | 166.80 ± 4.41 | 70.77 ± 1.75 |
| *n*-Butanol extract of Leaves | 2.80 | 883.99 ± 4.98 | 275.82 ± 2.20 | 118.47 ± 6.26 |

**Table 2**
Antioxidant and free radical scavenging potentiel (Percentage inhibition) of n-butanol extracts of C. fontanesii flowers and leaves in vitro.

| Samples | GOR scavenging activity (% of Inhibition) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3.125 µg/mL | 6.25 µg/mL | 12.5 µg/mL | 25 µg/mL | 50 µg/mL | 100 µg/mL | 200 µg/mL | 400 µg/mL |
| Flowers | na | 7.28 ± 0.23 | 22.00 ± 2.86 | 56.11 ± 1.79 | 63.12 ± 0.15 | nt | nt | nt |
| Leaves | na | 23.49 ± 0.43 | 54.69 ± 1.09 | 62.17 ± 1.18 | 62.88 ± 0.43 | nt | nt | nt |
| BHT | 39.11 ± 2.34 | 58.67 ± 0.94 | 69.65 ± 0.04 | 72.44 ± 0.23 | 73.61 ± 0.10 | nt | nt | nt |
| BHA | 46.67 ± 0.25 | 62.27 ± 1.40 | 71.46 ± 0.29 | 73.25 ± 0.41 | 73.78 ± 0.17 | nt | nt | nt |
| **ABTS (% of Inhibition)** | | | | | | | | |
| Flowers | 16.30 ± 2.10 | 30.76 ± 1.02 | 59.15 ± 3.31 | 92.24 ± 0.55 | 92.40 ± 1.24 | 92.51 ± 0.81 | 91.13 ± 0.79 | nt |
| Leaves | 29.44 ± 1.83 | 57.51 ± 1.29 | 91.98 ± 0.37 | 92.19 ± 0.51 | 92.35 ± 0.18 | 92.51 ± 0.37 | 90.98 ± 0.95 | nt |
| BHT | 59.22 ± 0.59 | 78.55 ± 3.43 | 90.36 ± 0.00 | 92.18 ± 1.27 | 93.37 ± 0.86 | 94.87 ± 0.87 | 96.68 ± 0.39 | nt |
| BHA | 83.42 ± 4.09 | 93.52 ± 0.09 | 93.58 ± 0.09 | 93.63 ± 0.16 | 93.63 ± 0.95 | 94.20 ± 0.90 | 95.39 ± 2.62 | nt |
| **OH (% of Inhibition)** | | | | | | | | |
| Flowers | na | na | 3.33 ± 0.80 | 14.07 ± 0.51 | 24.32 ± 2.37 | 31.55 ± 2.61 | 37.00 ± 0.59 | 55.57 ± 0.59 |
| Leaves | na | na | 4.21 ± 3.63 | 13.02 ± 0.37 | 36.26 ± 2.38 | 58.35 ± 1.12 | 65.68 ± 1.03 | 68.50 ± 2.48 |
| Ascorbic acid | na | 7.18 ± 2.62 | 15.83 ± 1.61 | 44.50 ± 3.34 | 75.23 ± 2.28 | 87.84 ± 1.78 | 88.37 ± 0.45 | nt |
| **Reducing power (% of Inhibition)** | | | | | | | | |
| Flowers | 0.22 ± 0.01 | 0.31 ± 0.03 | 0.48 ± 0.00 | 0.71 ± 0.04 | 0.90 ± 0.10 | 1.26 ± 0.07 | 1.34 ± 0.19 | nt |
| Leaves | 0.28 ± 0.03 | 0.38 ± 0.06 | 0.60 ± 0.03 | 0.84 ± 0.02 | 1.09 ± 0.10 | 1.35 ± 0.12 | 1.45 ± 0.12 | nt |
| Ascorbic acid | 0.35 ± 0.05 | 0.46 ± 0.03 | 0.84 ± 0.12 | 0.93 ± 0.30 | 1.18 ± 0.34 | 1.37 ± 0.20 | 1.44 ± 0.21 | nt |
| Tannic acid | 0.28 ± 0.02 | 0.78 ± 0.06 | 1.02 ± 0.07 | 1.24 ± 0.18 | 0.86 ± 0.6 | 1.01 ± 0.21 | 1.02 ± 0.13 | nt |
| α-Tocopherol | 0.11 ± 0.00 | 0.16 ± 0.00 | 0.21 ± 0.03 | 0.35 ± 0.03 | 0.73 ± 0.03 | 1.37 ± 0.08 | 1.81 ± 0.09 | nt |
| **CUPRAC (% of Inhibition)** | | | | | | | | |
| Flowers | 0.25 ± 0.01 | 0.36 ± 0.01 | 0.61 ± 0.01 | 1.07 ± 0.03 | 1.90 ± 0.07 | 3.20 ± 0.05 | 4.00 ± 0.04 | nt |
| Leaves | 0.41 ± 0.01 | 0.69 ± 0.03 | 1.21 ± 0.03 | 2.15 ± 0.09 | 3.40 ± 0.01 | 3.96 ± 0.11 | 3.90 ± 0.10 | nt |
| BHT | 0.19 ± 0.01 | 0.33 ± 0.04 | 0.66 ± 0.07 | 1.03 ± 0.07 | 1.48 ± 0.09 | 2.04 ± 0.14 | 2.32 ± 0.28 | nt |
| BHA | 0.46 ± 0.00 | 0.78 ± 0.01 | 1.34 ± 0.08 | 2.36 ± 0.17 | 3.45 ± 0.02 | 3.76 ± 0.03 | 3.93 ± 0.01 | nt |
| **Phenanthroline (% of Inhibition)** | | | | | | | | |
| Flowers | 0.35 ± 0.01 | 0.45 ± 0.00 | 0.61 ± 0.03 | 0.92 ± 0.02 | 1.62 ± 0.02 | 3.17 ± 0.11 | 4.17 ± 0.11 | nt |
| Leaves | 0.43 ± 0.01 | 0.63 ± 0.01 | 1.01 ± 0.04 | 1.91 ± 0.08 | 3.41 ± 0.11 | 4.11 ± 0.06 | 4.21 ± 0.05 | nt |
| BHT | 0.73 ± 0.02 | 0.93 ± 0.01 | 1.25 ± 0.04 | 2.10 ± 0.05 | 4.89 ± 0.06 | nt | nt | nt |
| BHA | 0.53 ± 0.03 | 1.23 ± 0.02 | 1.84 ± 0.01 | 3.48 ± 0.03 | 4.84 ± 0.01 | nt | nt | nt |
| **β-carotene (% of Inhibition)** | | | | | | | | |
| | **6.25 µg/mL** | **12.5 µg/mL** | **25 µg/mL** | **50 µg/mL** | **100 µg/mL** | **200 µg/mL** | **400 µg/mL** | **800 µg/mL** |
| Flowers | na | 0.12 ± 0.34 | 1.09 ± 0.98 | 2.32 ± 0.46 | 3.13 ± 1.53 | 6.02 ± 2.20 | 40.70 ± 2.95 | 62.27 ± 2.10 |
| Leaves | na | 1.52 ± 1.85 | 2.37 ± 0.94 | 3.13 ± 1.21 | 5.65 ± 0.28 | 34.74 ± 0.61 | 56.74 ± 0.25 | 63.13 ± 1.86 |
| BHT | 86.0.9 ± 1.04 | 88.29 ± 0.10 | 91.70 ± 0.36 | 93.65 ± 0.3 | 93.68 ± 0.46 | 94.49 ± 0.07 | 94.88 ± 0.10 | 95.58 ± 0.19 |
| BHA | 90.11 ± 0.68 | 93.48 ± 0.44 | 95.52 ± 0.33 | 96.34 ± 0.55 | 97.56 ± 0.19 | 97.64 ± 2.22 | 97.85 ± 0.32 | 99.66 ± 0.52 |
| **Metal chelating assays** | | | | | | | | |
| Flowers | na | 4.01 ± 1.71 | 7.63 ± 0.82 | 12.14 ± 1.13 | 17.66 ± 1.23 | 27.07 ± 0.19 | 39.46 ± 0.07 | 56.37 ± 0.33 |
| Leaves | na | 11.17 ± 2.80 | 16.15 ± 1.01 | 20.32 ± 1.64 | 24.49 ± 2.11 | 38.11 ± 1.02 | 40.77 ± 0.91 | 52.23 ± 0.32 |
| EDTA | | 73.00 ± 1.59 | 73.60 ± 1.20 | 73.80 ± 1.51 | 95.78 ± 0.10 | 95.80 ± 0.06 | 95.84 ± 0.22 | 95.87 ± 0.06 |

Values are expressed as means ± S.D of three parallel measurements. nt: not tested. na: not active.

than BHT and BHA.

The antioxidant activities using Phenanthroline assay of the extracts were also investigated as shown in Tables 2 and 3 The antioxidant activities of the *C. fontanesii* extracts were found to be 4.23 ± 0.15 and 8.37 ± 0.41 µg/mL for leaves and flowers, respectively.

The reductive potential of *C. fontanesii* extracts in reducing power assay is determined by the ability of the antioxidants in these extracts to reduce ferric to ferrous ion. The reduction properties are generally associated with the presence of reductones, which have the capacity to donate an electron to free radicals and convert them into more stable forms. The results of reducing potential are shown on Table 2. Leaves and flowers extracts showed good levels of electron donation abilities which were higher than that α-tocopherol and lower than that of ascorbic acid and tannic acid which are well known as a strong reducer. The obtained results for CUPRAC assay indicate high activities of *C. fontaneisi* extracts (Tables 2 and 3) which exhibited a similar activity than the synthetic antioxidant BHT or BHA. Leaves extract (4.10 ± 0.15 µg/mL) was the most active with a similar activity to that of BHA (3.64 ± 0.19 µg/mL) evidencing a promising health-promoting value of analyzing extracts.

Moreover, the antioxidant activity of leaves and flowers extracts was tested also towards hydroxyl radical, a high reactive oxygen species (capable to attack most biological substates including, DNA, carbohydrates, proteins and polyunsaturated fatty acids) and on the inhibition of lipid peroxidation (β-carotene test). The extracts radical scavenging activity vs. hydroxyl radical was expressed as $IC_{50}$, and leaves extract

**Table 3**
Antioxidant activities ($IC_{50}$ μg/mL) of *n*-butanol extracts of *C. fontanesii* flowers and leaves.

| Samples | Radical scavenging activity $IC_{50}$ (μg/mL) | | | Reducing power $A_{0.5}$ (μg/mL) | | | Antioxidant $IC_{50}$ (μg/mL) | Metal chelating assays |
|---|---|---|---|---|---|---|---|---|
| | GOR | ABTS | OH | CUPRAC | FRAP | Phenanthroline | β-carotene | Ferrous chelating) |
| Leaves | 8.49 ± 0.59$^{b}$ | 5.25 ± 0.21$^{b}$ | 81.01 ± 3.18$^{b}$ | 4.10 ± 0.15$^{b}$ | 9.61 ± 1.17$^{c}$ | 4.23 ± 0.15$^{b}$ | 276.12 ± 3.63$^{b}$ | 726.25 ± 5.00$^{a}$ |
| Flowers | 15.35 ± 0.28$^{a}$ | 10.32 ± 0.88$^{a}$ | 340.08 ± 3.93$^{a}$ | 9.66 ± 0.26$^{a}$ | 14.73 ± 1.11$^{b}$ | 8.37 ± 0.41$^{a}$ | 513.80 ± 11.04$^{a}$ | 655.31 ± 5.07$^{b}$ |
| BHT | 4.99 ± 0.06$^{c}$ | 1.29 ± 0.30$^{c}$ | – | 9.62 ± 0.87$^{a}$ | – | 2.24 ± 0.17$^{c}$ | 0.91 ± 0.01$^{c}$ | – |
| BHA | 4.35 ± 0.13$^{c}$ | 1.81 ± 0.10$^{c}$ | – | 3.64 ± 0.19$^{b}$ | – | 0.93 ± 0.07$^{d}$ | 1.05 ± 0.03$^{c}$ | – |
| Ascorbic acid | – | – | 32.33 ± 1.17$^{c}$ | – | 6.77 ± 1.15$^{d}$ | – | – | – |
| Tannic acid | – | – | – | – | 5.39 ± 0.91$^{d}$ | – | – | – |
| α-Tocopherol | – | – | – | – | 34.93 ± 2.38$^{a}$ | – | – | – |
| EDTA | – | – | – | – | | – | – | 8.80 ± 0.47$^{c}$ |

Values are expressed as means ± S.D. of three parallel measurements. Means with different superscript letters in the same column are significantly ($P < 0.05$) different.

showed the highest antioxidant activity having the lowest value of $IC_{50}$ (81.01 ± 3.18 μg/mL); flowers reported the highest $IC_{50}$ values (340.08 ± 3.93 μg/mL). Interestingly, these values were higher than ascorbic acid used as standard ($IC_{50}$ 32.33 ± 1.17 μg/mL).

In β-carotene assay we measures the potentiel of *n*-butanol extracts of *C.fontanesii* leaves and flowers for inhibiting conjugated diene hydroperoxides formation via linoleic acid oxidation. The presence of antioxidants minimizes the oxidation of β-carotene (Braca et al., 2018). The inhibition effect of these extracts is presented in Tables 2 and 3 From this study, oxidation of β-carotene was effectively inhibited by the tow extracts which showed moderate inhibition compared with BHT and BHA (the reference compound). However, *n*-butanol extract of *C. fontanesii* leaves reported the highest β-carotene bleaching inhibition activity (276.12 ± 3.63 μg/mL) compared to flowers extract (513.80 ± 11.04 μg/mL) (Table 3). Since transition metals can accelerate the lipid peroxidation via the Fenton reaction, the metal chelating activity was selected to measure the binding capacity of iron of the extracts. The chelating effects of the extracts on ferrous ions, compared with EDTA is dose dependant (Table 3). The *n*-butanol extract of flowers ($IC_{50}$ value: 655.31 ± 5.07 μg/mL) showed the highest metal chelating activity compared to leaves extract ($IC_{50}$ value: 726.25 ± 5.00 μg/mL) which were significantly higher than that EDTA ($IC_{50}$ value: 8.80 ± 0.47 μg/mL).

### 3.5. Enzyme inhibition

Enzyme inhibitory effects of the of *n*-butanol extracts from leaves and flowers of *C. fontanesii* were investigated toward a panel of important enzymes such as acetylcholinesterase (AChE). Butyrylcholinesterase (BChE), α-glucosidase and tyrosinase. The results are presented in Table 4. Generally, from the enzymatic standpoint, the best inhibitory effects were observed in the leaves extract. Except for the lack of inhibitory effect on AChE and BChE. These variations observed can be explained by the different phytochemical compositions of the *C. fontanesii* extracts.

The inhibitory activities of the extracts on AChE and BChE were reported as potent (> 50%), moderate (30–50%), inactive, or low (< 30%) activity (Vinutha et al., 2007). According to this classification, the *n*-butanol extracts from leaves and flowers of *C. fontanesii* showed low inhibitory activity against AChE (Table 4). On the other hand, the *C. fontanesii* extracts remained inactive.

The α-glucosidase inhibitor effects of *n*-butanol extracts from leaves and flowers of *C. fontanesii* are presented in Table 4. The low $IC_{50}$ values designated the high inhibition activity. Thus, the greatest α -glucosidase inhibition activity was obtained in *n*-butanol extract of leaves, with $IC_{50}$ of 83.20 ± 13.54 μg/mL. *n*-butanol extract of flowers also exhibited good α-glucosidase inhibition ($IC_{50}$ = 142.16 ± 17.91 μg/mL). This biological activity was better than that of acarbose ($IC_{50}$ = 275.43 ± 1.59 μg/mL) ($P < 0.05$).

### 3.6. Dermatoprotective effects

The Photoprotective and tyrosinase inhibitory activities were used to evaluate the dermatoprotective effects.

#### 3.6.1. Tyrosinase inhibition

In the current study, both kojic acid and *n*-butanol extract of flowers of *C. fontanesii* inhibited tyrosinase activity in a dose-dependent manner, while the leaves extract showed no activity (Table 4). The observed tyrosinase inhibitory activity of *n*-butanol extract of flowers was more potent to that of kojic acid ($P < 0.05$), a well-known tyrosinase inhibitor and whitening agent (Table 4).

#### 3.6.2. Photoprotective activity

The photoprotective activity of plant extracts was measured by determining in vitro of sun protection factor (SPF), which is considered to be one of the most frequently used indicators for the classification of protection levels afforded by sunscreen products against sunburn due mainly to harmful UV-B radiation (Miksa et al., 2015). It has been reported that in SPF ratings, SPF values 2–12, 12–30, 30–50 and >50 are

**Table 4**
Percentage enzyme inhibition and $IC_{50}$ (μg/mL) of *n*-butanol extracts of *C. fontanesii* flowers and leaves.

| Samples | AChE | | BChE | | α-glucosidase | | Tyrosinase | |
|---|---|---|---|---|---|---|---|---|
| | % Inhibition at 200 μg/mL | $IC_{50}$ (μg/mL) | % Inhibition at 200 μg/mL | $IC_{50}$ (μg/mL) | % Inhibition at 500 μg/mL | $IC_{50}$ (μg/mL) | % Inhibition at 200 μg/mL | $IC_{50}$ (μg/mL) |
| Leaves | 12.34 ± 9.84 | < 200 | 17.08 ± 4.30 | < 200 | 82.69 ± 3.88 | 83.20 ± 13.54$^{c}$ | na | na |
| Flowers | 7.31 ± 2.60 | < 200 | 21.24 ± 6.86 | < 200 | 75.25 ± 0.90 | 142.16 ± 17.9$^{b}$ | 54.71 ± 1.46 | 21.89 ± 0.41$^{b}$ |
| Galantamine | 94.77 ± 0.34 | 6.27 ± 1.15 | 78.95 ± 0.58 | 34.75 ± 1.99 | – | – | – | – |
| Acarbose | – | – | – | – | 60.69 ± 2.29 | 275.43 ± 1.59$^{a}$ | – | – |
| Kojic acid | – | – | – | – | – | – | 66.95 ± 2.24 | 25.23 ± 0.78$^{a}$ |

Values are expressed as means ± S.D of three parallel measurements. Means with different superscript letters in the same column are significantly ($P < 0.05$) different. na: not active.
Galantamine is control for Acetylcholinesterase (AChE). Butrylcholinesterase (BChe). Acarbose for α-glucosidase and Kojic acid for Tyrosinase.

considered as having respectively minimum, moderate, high and maximum sun protective activity (Schalka et al., 2011). In this study the SPF value was found to be 38.96 ± 0.26 and 38.66 ± 0.68 for leaves and flowers, respectively. The results indicate that the *n*-butanol extracts have displayed high photoprotective activity and can be employed in sunscreen formulations to protect the skin from sunburn.

Photoprotective and enzyme inhibitory activity of *C. fontanesii* has not previously been reported elsewhere. Therefore, data presented in this communication could be the first report for the literature.

## 4. Discussion

Antioxidative effects of natural products could be considered as a first insight in detecting their ethnopharmacological relevance and potential. To this end, a certain antioxidant profile could be provided by comparing different chemical assays representative of alternative mechanisms (Zengin et al., 2018). For this reason, the antioxidant ability of *C. fontaneisi* was evaluated using eight complementary in vitro tests: free-radical scavenging (GOR, ABTS and $OH^{\cdot}$), reducing power, CUPRAC, phenanthroline, β-carotene and metal chelating assays. The results are summarized in Tables 1 and 2 The *n*-butanol extract from leaves exhibited stronger free-radical scavenging, antioxidant and reducing potential when compared to the flowers extract. However, the best activity in metal chelating assays was obtained by the flowers extract. The higher radical scavenging capacity of leaves extract of the genus *Chrysanthemum* is supported by previous studies and can be attributed to the highest values in terms of phenolics and flavonoids presented by this extract (Wei et al., 2015; Alexieva et al., 2013).

Enzymes involved in some key metabolic processes are considered a potential therapeutic target for controlling public health problems (Vujanovića et al., 2019).

Tyrosinase is known to be a key enzyme in melanin biosynthesis, involved in determining the color of mammalian skin, hair and eyes providing the protection against UV. In addition, unfavorable enzymatic browning of plant – derived food by tyrosinase cause a decrease in nutritional quality and economic loss of food products (Kim and Uyama, 2005). Over expression of tyrosinase also involved in the pathogenesis of parkinson's disease, oxidizing excess dopamine to produce dopamine quinones, highly reactive and neuron-specific cytotoxic molecules which induce neural damage and cell death (Saghaie et al., 2013). Herbal medicines provide an interesting, largely unexplored source for the development of potential new agents that can antagoniste tyrosinase activity. Many plant extracts are known to be inhibitors of tyrosinase, sometimes more potent than the standard inhibitors, arbutin or kojic acid, and without side effects (Kamagaju et al., 2013). In this study, the extracts from *C. fontanesii* leaves and flowers were evaluated for their tyrosinase inhibitory activities. The flowers extract showed the maximum tyrosinase inhibitory activity ($IC_{50}$: 21.89 ± 0.41 μg/mL) and was more potent than kojic acid ($IC_{50}$: 25.23 ± 0.78 μg/mL). The tyrosinase inhibitory activity associated with the antioxidant effects of flowers extract of *Chrysanthemum* species is supported by previous studies (Choi et al., 2016; Kim et al., 2018).

High level of carbohydrate intake is inevitably associated with an increased risk for obesity and type 2 diabetes. α-Amylase and α-glucosidase are two classes of the enzymes responsible for carbohydrate digestion resulting in postprandial high glucose level in the body (Habtemariam and Varghese, 2014). The use of carbohydrate digestion enzyme inhibitors from natural resources could be a possible strategy to block dietary carbohydrate absorption and present an economical alternative to the oral synthetic hypoglycemic drugs with less adverse effects (Kandouli et al., 2017). Several studies on plant extracts of *Chrysanthemum* genus belongs to the family of Asteraceae that may contribute to diabetes treatment have focused on the inhibition of α-glucosidase. which catalyze carbohydrate digestion into glucose (Yang et al., 2011; Thi Luyen et al., 2013; Ben Sassi et al., 2018). In this study, we investigated the inhibitory effect of *C. fontanesii* extracts on α-glucosidase to elucidate the potential of *C. fontanesii* as a natural agent in reducing postprandial hyperglycemia. *C. fontanesii* extracts exhibited a significantly higher inhibitory effect than acarbose on α-glucosidase, which suggested that *C. fontanesii* extracts could control diabetes by reducing postprandial hyperglycemia.

The use of sunscreen as photoprotecting agents for UV protection is becoming very popular. Its function is based on its ability to absorb, reflect or scatter the sun's rays. The Sun protection factor (SPF) is one of the most frequently used indicators for the classification of protection levels afforded by sunscreen products against sunburn. Higher SPF sunscreens offer greater protection from sunburn (Mishra and Chattopadhyay, 2011). In this study, *n*-butanol extracts of *C. fontaneisii* have displayed high photoprotective activity and can be employed in sunscreen formulations to protect the skin from sunburn.

## 5. Conclusion

This study, for the first time, showed that *C. fontanesii* has interesting photoprotective, tyrosinase and α-glucosidase inhibitory activities. Therefore, *C. fontanesii* may be interesting for the treatment of diabetes mellitus and skin disorders not only by inhibiting an enzyme that is related with the disorder, but also by improving the antiradical defenses of the patients. According to our findings, *C. fontanesii* has a great importance in the medical, cosmetic and food industries.

## Conflicts of interest

The authors declare no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101209.

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