Antioxidant and α-glucosidase activities and phytochemical constituents of *Chrysanthoglossum trifurcatum* (Desf.)

Ahlem Ben Sassi¹, Sana Amroussi¹, Malek Besbes¹, Mahjoub Aouni¹, Fethia Skhiri²

¹Laboratory of Transmissible Diseases and Biologically Active Substances, Department of Microbiology, Faculty of Pharmacy, University of Monastir, Tunisia
²Laboratory of Genetics, Biodiversity and Valorisation of Bioresources, Higher Institute of Biotechnology, University of Monastir, Tunisia

**Abstract**

**Objective:** To investigate the antioxidant and α-glucosidase properties and phytochemical constituents of roots, stems, leaves and flowers extracts and aerial parts oil of *Chrysanthoglossum trifurcatum* (Desf.) (*C. trifurcatum*). **Methods:** For extraction from roots, stems, leaves and flowers of *C. trifurcatum*, methanol, ethyl acetate and petroleum ether solvents were used. Phenols, flavonoids, flavonols and tannins contents were evaluated. More, *C. trifurcatum* aerial parts oil composition was determined using chromatography/mass spectrometry. The antioxidant effect was estimated by DPPH, ABTS and reducing power test systems. The α-glucosidase inhibition was determined by colorimetric assay using the enzyme from *Aspergillus niger* and the p-nitrophenyl glucopyranoside (pNPG) as substrate. **Results:** The highest amounts of polyphenols, flavonoids, flavonols and tannins were shown by the methanolic extract of leaves. The main components of the aerial parts oil were limonene (29.21%), γ-terpinene (12.96%), 4-terpenyl acetate (12.18%) and α-pinene (5.76%). The activity evaluated by DPPH, ABTS and reducing power tests was important for stems (IC₅₀=0.68 mg/mL) and flowers (IC₅₀=0.67 mg/mL) methanolic extracts and essential oil (IC₅₀=0.72 mg/mL). Findings of α-glucosidase activity revealed that petroleum ether extracts of leaves and roots together with aerial parts oil showed a highest activity with IC₅₀ of 0.044, 0.045 and 0.049 mg/mL, respectively. **Conclusions:** Observed antioxidant and α-glucosidase activities of oil and extracts are attributed to the presence of the active phytochemicals in *C. trifurcatum* organs. Thus, the *C. trifurcatum* can be used as a source of antioxidant compounds and dietary supplement to treat patients with type 2 diabetes.

1. Introduction

Many medicinal and aromatic plants contain antioxidants such as phenols. These components can have a significant role in the neutralization and absorption of radicals and peroxide decomposition or extinction. Many phenolic constituents for example flavonoids and phenolic acids have antioxidant potential thus they contribute significantly to the fight against many human diseases and contribute to the mortality reduction[1].

The type 2 diabetes mellitus is one of the common metabolic diseases. Treatment of type 2 diabetes improves patient blood sugar levels and insulin secretion stimulated by drugs such as α-glucosidase inhibitors and metformin[2]. α-glucosidase serves to inhibit postprandial hyperglycemia and reduce the glucose absorption, and it is located in small intestine epithelium. Several α-glucosidase inhibitors for example acarbose, voglibose and miglitol[3] are clinically used. Thus, the molecules of plant origin represent a source of such inhibitors.
The *Chrysanthemum* genus belongs to the family of Asteraceae which is common in countries of Mediterranean basin\(^4,5\). We counted 13 *Chrysanthemum* species distributed in many regions in Tunisia\(^6\). *Chrysanthoglossum trifurcatum* (Desf.) (synonym of *Chrysanthemum trifurcatum* Desf. var. *macrocephalum* (Viv.) Beg.)\(^7\) (*C. trifurcatum*) is largely disseminated in Tunisian regions. It is used to treat problems of intestinal transit, constipation and postdelivery pains\(^8\). It has been reported that extract with methanol solvent of Tunisian *C. trifurcatum* stimulates the contractions of duodenal smooth muscles through muscarinic receptors\(^9\). The same authors reported that the hot water, methanol, ethyl acetate and petroleum extracts and essential oils of *C. trifurcatum* exhibited activity against many bacteria and yeasts\(^8,9\). On other hand, the same extracts and oils have a lower effect against Herpes simplex virus type-1. Recent findings have reported that flavonoids are the most important phytocomponents of methanol extracts of leaves, stems and flowers\(^10\). Other researchers have identified five flavonoids and one phenolic acid (caffeic acid) in the butanolic fraction of Algerian *C. trifurcatum*, which had an antioxidant activity\(^11\).

However, no studies have been conducted to determine the antioxidant and α-glucosidase activities of *C. trifurcatum* growing in Tunisia. Thus, the research objectives are to evaluate reducing power, scavenging ability of ABTS and DPPH radicals, α-glucosidase inhibitor activity, total phenols, flavonoids, flavonols and tannins content of the methanolic, ethyl acetate and petroleum ether extracts of roots, leaves, stems and flowers and the composition of aerial parts oil of *C. trifurcatum*.

### 2. Materials and methods

#### 2.1. *C. trifurcatum* material

Plant material was collected in March 2015 (in flowering stage) from Monastir area in centre of Tunisia. Plant was botanically identified by Pr. Skhiri Fethia (High Institute of Biotechnology, Monastir University, Tunisia) according to the description of morphology existing in Flora of Tunisian\(^6\). The fresh material (roots, stems, leaves, flowers and aerial parts) was air-dried in shadow for 10 d and powdered.

#### 2.2. Organic extracts preparation

The material of *C. trifurcatum* organs (100 g) was extracted using three increasing polarity solvents: petroleum ether, ethyl acetate, and methanol. Twelve obtained extracts were evaporated and yields were determined.

#### 2.3. Phenolic content determination

##### 2.3.1. Total polyphenolic content

Total phenolic content was measured by Folin–Ciocalteu method\(^12,13\). Indeed, sample (1 mg/mL, 50 μL) and water/Folin–Ciocalteu solution [28:2 (v/v), 750 μL] were mixed. Sodium carbonate (20%, 200 μL) was added after 3 min. After incubation in a boiling water bath for 1 min, the mixture was kept for 30 min in the dark. Methanol was used as the control sample. Absorbance was determined at 765 nm. Total phenols were evaluated by relating the absorbance in the calibration curve prepared with solutions of gallic acid ranging from 0 to 250 μg/mL (\(r=0.99\)). Results are reported as mg of gallic acid equivalent/100 g of dry weight (mg GAE/100 g DW).

##### 2.3.2. Flavonoid content

Flavonoid content of the different plant organs was evaluated by the aluminum chloride method\(^1\). Diluted sample (0.5 mL) was added to aluminum chloride solution (2%, 0.5 mL). Mixture was incubated for 15 min at ambient temperature, and then absorbance was measured at 430 nm. Catechin calibration curve ranging from 0 to 250 μg/mL (\(r=0.99\)) was used. Content of flavonoids is presented as mg catechin equivalent/100 g of dry weight (mg CE/100 g DW).

##### 2.3.3. Flavonol content

The flavonol contents were estimated using aluminum chloride method\(^14\). Extract (1 mg/mL, 1 mL) was added to aluminum chloride (2%, 1 mL) and sodium acetate (5%, 3 mL) solutions. After incubating the mixture at ambient temperature for 2.5 h, absorbance was determined at 440 nm. Catechin with calibration curve ranging from 0 to 250 μg/mL (\(r=0.99\)) was used. The content of flavonols is presented as mg catechin equivalent/100 g of dry weight (mg CE/100 g DW).

##### 2.3.4. Tannins content

Tannins were evaluated by the method of Sun *et al.*\(^15\) with minor modifications. Thus, vanillin (4%, 3 mL) and concentrated H\(_2\)SO\(_4\) (1.5 mL) solutions were mixed with diluted extract (50 μL). Absorbance was determined at 500 nm after 15 min. Tannin content is expressed as mg catechin equivalent/100 g of dry weight (mg CE/100 g DW). Catechin calibration curve ranged from 0 to 400 μg/mL (\(r=0.99\)).

#### 2.4. Essential oil isolation

The fresh aerial parts material was submitted for 5 h for hydrodistillation. Essential oil yield was determined based on sample fresh weight.

#### 2.5. Chromatographic analysis

Gas chromatography-mass spectrometer (GC–MS) analyses of aerial parts oil were performed as previously reported\(^16\).

#### 2.6. Antioxidant activity evaluation

Antioxidant effect of *C. trifurcatum* oil and extracts was measured by reducing power and DPPH and ABTS free radical-scavenging assays.
2.6.1. DPPH radical scavenging assays

Anti-radical activity was evaluated using the modified method of Ramadan et al.[17]. Thus, DPPH (950 μL, 1 × 10−5 mol/L) was mixed with diluted extract (50 μL). After 30 min, the mixture absorbance was read at 515 nm. Trolox was the positive control. The anti-radical activity was determined by the formula:

\[ \% \text{ inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \]

Where, \( A_{\text{control}} \) is control reaction absorbance, and \( A_{\text{sample}} \) is sample test absorbance. Thereafter, IC50 value (concentration responsible to inhibit DPPH radicals to 50%) was determined.

2.6.2. ABTS radical scavenging assays

Re et al. method[18] with minor modification was used to evaluate anti-radical activity. Thus, ABTS (7 mmol/L) were mixed with potassium ferric cyanide (2.45 mmol/L). To a diluted ABTS− solution (990 μL), 10 μL of the sample (1 mg/mL) were added. After 20 min, the absorbance was determined at 734 nm. Trolox is the positive control and anti-radical activity was determined by the following formula:

\[ \% \text{ inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \]

Where, \( A_{\text{control}} \) is control reaction absorbance, and \( A_{\text{sample}} \) is sample test absorbance. IC50 value (concentration responsible to inhibit ABTS radicals to 50%) was determined.

2.6.3. Reducing power

The modified Sanja et al. method[19] was adopted to evaluate the ferric-reducing power of oil and extracts. Sample solutions (0.5–3.0 mg/mL) were mixed with potassium peroxodisulfate (2.45 mmol/L). To a diluted ABTS− solution (990 μL), 10 μL of the sample (1 mg/mL) were added. After 20 min, the absorbance was determined at 734 nm. Trolox is the positive control and anti-radical activity was determined by the formula:

\[ \% \text{ inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \]

Where, \( A_{\text{control}} \) is control reaction absorbance, and \( A_{\text{sample}} \) is sample test absorbance. IC50 value (concentration responsible to inhibit ABTS radicals to 50%) was determined.

2.7. α-glucosidase inhibitory activity

The α-glucosidase inhibition effect was evaluated using the Tao et al. method[20]. Thus, p-nitrophenyl-α-D-glucopyranoside (2.5 mmol/L) was mixed with α-glucosidase (0.3 U/mL) and extract or oil in DMSO (250 μL). The reaction mixture was incubated at 37 °C for 15 min. Acarbose was a standard. Absorbance was measured at 405 nm. Inhibition ratio (%) by samples and acarbose were determined by the equation:

\[ \% \text{ inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \]

Where, \( A_{\text{control}} \) is sample absorbance and \( A_{\text{sample}} \) is control absorbance. The concentration of the sample that inhibits the enzyme by 50% was evaluated.

2.8. Statistical analysis

Results are expressed as mean ± SD from three separate observations. Linear regression analysis was adopted to determine the IC50 (DPPH, ABTS and reducing power methods) values. Data were analyzed by SPSS software (SPSS v.20) and Duncan test was used to determine statistical significance (P<0.05 deemed as significant).

3. Results

3.1. Yields and phenol contents

Yields of extractions from flowers, stems, leaves and roots of C. trifurcatum by varying polarity solvents (methanol, ethyl acetate and petroleum) are represented in Table 1. The highest yield was obtained by methanolic extraction. In different plant parts, the yields of methanolic extracts were 0.91% for roots, 1.10% for stems, 5.10% for flowers and 6.20% for leaves. The contents of phenol, flavonoid, flavonol and tannin varied significantly with type of plant material; mg CE/100 g DW: mg catechin equivalents per 100 g of dry weight of the plant material.

| Organ     | Solvent extraction | Yield (%) | Total phenols (mg GAE/100 g DW) | Total flavonoids (mg CE/100 g DW) | Flavonols (mg CE/100 g DW) | Tannins (mg CE/100 g DW) |
|-----------|--------------------|-----------|---------------------------------|-----------------------------------|---------------------------|-------------------------|
| Flowers   | Petroleum ether    | 1.69      | 1.57±0.35                      | 92.84±8.78                       | 15.34±0.65                | 5.39±0.10               |
|           | Ethyl acetate      | 0.60      | 1.59±0.33                      | 46.62±1.05                       | 5.51±1.46                 | 0.05±0.002              |
|           | Methanol           | 5.10      | 20.85±2.68                     | 634.81±1.39                      | 129.95±1.07               | 2.12±0.06               |
| Leaves    | Petroleum ether    | 2.92      | 12.56±2.96                     | 522.43±2.86                      | 357.32±1.66               | 4.87±0.30               |
|           | Ethyl acetate      | 1.69      | 40.59±4.08                     | 378.37±10.41                     | 505.99±1.05               | 4.67±0.20               |
|           | Methanol           | 6.20      | 142.51±1.29                    | 625.83±7.89                      | 624.13±1.18               | 11.92±0.20              |
| Stems     | Petroleum ether    | 0.29      | 0.27±0.02                      | 13.44±3.55                       | 12.77±1.18                | 0.34±0.03               |
|           | Ethyl acetate      | 0.50      | 1.69±0.37                      | 151.07±1.20                      | 16.92±1.88                | 0.44±0.01               |
|           | Methanol           | 1.10      | 3.20±0.46                      | 95.27±1.32                       | 61.35±1.85                | 1.15±0.04               |
| Roots     | Petroleum ether    | 0.42      | 6.18±0.68                      | 107.46±2.50                      | 10.73±0.62                | 0.31±0.01               |
|           | Ethyl acetate      | 0.52      | 5.45±0.20                      | 57.52±2.04                       | 8.24±0.61                 | 0.30±0.01               |
|           | Methanol           | 0.91      | 7.86±0.53                      | 99.76±2.95                       | 65.92±3.5                 | 0.42±0.05               |

Means in each column followed by different letters are significantly different (P<0.05). mg GAE/100 g DW: mg gallic acid equivalents per 100 g of dry weight of the plant material; mg CE/100 g DW: mg catechin equivalents per 100 g of dry weight of the plant material.
and flavonoids. Ethyl acetate flower extract presented lower level of flavonols and tannins.

### 3.2. Essential oil composition

*C. trifurcatum* oil with the yield of 0.061% (w/w) was analyzed by GC–MS. As can be seen in Table 2, 44 constituents, representing 98.95% of oil, were detected. Limonene (29.21%), γ-pinene (12.96%), 4-terpenyl acetate (12.18%) and α-pinene (5.76%) were the main compounds in oil.

### 3.3. Antioxidant activity

The various extracts of *C. trifurcatum* exhibited remarkable antioxidant activities (Table 3). The radical scavenging and reducing power activities, expressed as inhibition percentage and IC₅₀, varied with type of solvent and plant part (Table 3).

By DPPH radical scavenging test, the best activity was observed with methanolic extracts for stems and flowers, and the lowest activity was observed in petroleum ether extract for stems. By ABTS radical scavenging test, the best activity was observed in methanol extract for stems and the lowest activity was observed in ethyl acetate extract for leaves. The results by reducing power test presented in Table 3 show that most extracts had some antioxidant activity and that the greatest activity was obtained with methanol for stems and flowers. Also, these activities were lower than a Trolox with IC₅₀ of 0.136, 0.161 and 0.082 mg/mL by DPPH, ABTS and reducing power tests, respectively. The results of antioxidant activity of aerial parts oil are presented in Table 3. Oil had an antioxidant activity with IC₅₀ of (0.72±0.01), (0.87±0.03) and (0.92±0.09) mg/mL by DPPH and ABTS radicals scavenging and reducing power assays, respectively. This activity was lesser than that of Trolox (Table 3).

### 3.4. α-glucosidase inhibition activity

The α-glucosidase inhibitor effectiveness of different *C. trifurcatum* extracts and oil are presented in Table 4. The low IC₅₀ values designated the high inhibition activity. Thus, the greatest α-glucosidase inhibition activity was obtained in petroleum ether extracts of leaves and roots and aerial parts oil, with IC₅₀ of 0.044, 0.045 and 0.049 mg/mL, respectively. Ethyl acetate extracts of stems (IC₅₀=0.054 mg/mL) and flowers (IC₅₀=0.057 mg/mL) also exhibited α-glucosidase inhibition, as well as methanolic stem extract (IC₅₀=0.064 mg/mL). This biological activity was better than that of acarbose (IC₅₀=0.07 mg/mL).

| Table 2 |
| Constituents of the essential oil from the aerial parts of *C. trifurcatum* (Desf.). |
| **Compound** | **Rf** | **Content (%)** | **Identification** |
| --- | --- | --- | --- |
| α-Hexenal | 860 | 2.35 | GC–MS, RI |
| Tricyclene | 930 | 1.63 | GC–MS, RI |
| α-Thujene | 935 | 3.97 | GC–MS, RI |
| α-Pinene | 940 | 5.76 | GC–MS, RI |
| Camphene | 952 | 0.12 | GC–MS, RI |
| Sabine | 976 | 1.21 | GC–MS, RI |
| β-Pinene | 979 | 2.71 | GC–MS, RI |
| β-Myrcene | 991 | 0.76 | GC–MS, RI |
| Limonene | 1032 | 29.21 | GC–MS, RI |
| γ-Terpene | 1064 | 12.96 | GC–MS, RI |
| α-Terpinolene | 1089 | 1.02 | GC–MS, RI |
| 1-Octen-3yl-acetate | 1096 | trd | GC–MS, RI |
| Camphor | 1145 | 0.36 | GC–MS, RI |
| Borneol | 1169 | 0.24 | GC–MS, RI |
| p-Cymen-8-ol | 1187 | 0.79 | GC–MS, RI |
| α-Terpineol | 1191 | 0.78 | GC–MS, RI |
| Myrtenal | 1193 | 0.81 | GC–MS, RI |
| Myrtenol | 1196 | 1.72 | GC–MS, RI |
| Fenchyl acetate | 1225 | 0.46 | GC–MS, RI |
| Carvacrol | 1291 | 1.06 | GC–MS, RI |
| 4-terpenyl acetate | 1340 | 12.18 | GCMS, RI |
| α-Cubebene | 1352 | 1.14 | GC–MS, RI |
| α-Terpenyl acetate | 1354 | 3.68 | GC–MS, RI |
| α-Ylangene | 1372 | trd | GC–MS, RI |
| α-Copaene | 1379 | 1.24 | GC–MS, RI |
| β-Bourbene | 1381 | 1.13 | GC–MS, RI |
| β-Gurjunene | 1409 | 2.05 | GC–MS, RI |
| β-Caryophyllene | 1420 | 0.18 | GC–MS, RI |
| β-Gurjunene | 1434 | 0.25 | GC–MS, RI |
| α-Cedrene | 1437 | 1.07 | GC–MS, RI |
| α-Himachalene | 1447 | 1.24 | GC–MS, RI |
| β-Farnesene | 1458 | 1.11 | GC–MS, RI |
| Germacrene-D | 1484 | 0.19 | GC–MS, RI |
| β-Selinene | 1489 | 0.26 | GC–MS, RI |
| α-Muurolone | 1502 | trd | GC–MS, RI |
| β-Bisabolene | 1508 | trd | GC–MS, RI |
| δ-Cadinene | 1523 | 1.09 | GC–MS, RI |
| Elemol | 1549 | 0.53 | GC–MS, RI |
| Germacrene-B | 1555 | 0.18 | GC–MS, RI |
| β-Calacorene | 1561 | 0.94 | GC–MS, RI |
| β-Spathulenol | 1576 | 0.16 | GC–MS, RI |
| τ-Cadinol | 1640 | 2.29 | GC–MS, RI |
| τ-Muurolol | 1642 | 0.12 | GC–MS, RI |
| α-Cadinol | 1654 | trd | GC–MS, RI |
| Total identified | | 98.95 | |
| Yield (%)(w/w) | | 0.06 | |

GC: Gas chromatography; MS: Mass spectrometry; RI: Retention indices.

The compounds are listed according to their elution order on the apolar HP-5MS capillary column, and their identification was based on the comparison of their mass spectra and RI with those reported in the Wiley 275L mass spectral library (6th edition) and in the literature [21-25]. *RI relatives to n-alkanes (C9-C28) on the apolar HP-5MS column. The contents (%) were calculated by electronic integration of the FID peaks areas obtained on the apolar HP-5MS column; trd: Trace (<0.05%).
The high content of solvent will play an important role in increasing solubility of better solvent in extracting polyphenols. Therefore, the polarity of solvent is important to containing several flavonoids[29]. In the previous studies, the most important phytochemicals of methanol extracts of flowers, leaves and stems of C. trifurcatum were flavonoids[10]. Isoharnnetin was identified in flowers, leaves and stems of C. trifurcatum. In contrast, flavones, flavonols and phenolic acid (cafeic acid) were present in flowers and leaves[10]. Mokaddem-Daroui et al. have reported that the butanolic fraction of Algerian C. trifurcatum contained flavonoids phenolic acid (cafeic acid)[11].

Aerial parts oil composition is somewhat similar to the compositions described in previous reports on C. trifurcatum leaves and stem oils[16]. Thus, the oil of leaves contained 41 constituents, representing 97.84% of oil and main components were limonene, γ-terpinene, α-pinene, α-terpenyl acetate and α-thujene. In the stem oil, 29 compounds were found and representing 99.02% of oil. The main components were limonene, 4-terpenyl acetate, γ-terpinene and 2-hexenal[16].

We deduced that the higher antioxidant activity was obtained with polar extracts. The antioxidant effects of C. trifurcatum may be related to its richness on phenols and flavonoids. Researchers have reported that the butanolic extract of C. trifurcatum may be rich in flavonoid-like luteolin and phenolic acid like caffeeic acid[10]. It is shown that the caffeeic acid is a potent radical scavenger and it is more effective than Trolox[35]. Luteolin is an important component in Chrysanthemum genus such Chrysanthemum morifolium[30], Chrysanthemum coronarium[31], Chrysanthemum indicum[32], Chrysanthemum balsamita[33] and Chrysanthemum fuscum[34]. However, Tahri et al. reported that methanol extracts of C. trifurcatum were rich in flavonoid-like luteolin and phenolic acid like cafeic acid[10]. It is shown that the caffeeic acid is a potent radical scavenger and it is more effective than Trolox[35].

Researchers have reported that Chrysanthemum species were found to contain several flavonoids[29]. In the previous studies, the most important phytochemicals of methanol extracts of flowers, leaves and stems of C. trifurcatum were flavonoids[10]. Isoharnnetin was identified in flowers, leaves and stems of C. trifurcatum. In contrast, flavones, flavonols and phenolic acid (cafeic acid) were present in flowers and leaves[10]. Mokaddem-Daroui et al. have reported that the butanolic fraction of Algerian C. trifurcatum contained flavonoids phenolic acid (cafeic acid)[11].

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the antioxidant potential of *C. trifurcatum* can be attributed to the existing of these constituents in different plant parts. In addition, aerial parts oil of this specie was rich in limonene and α-pinene, which are known as potent anti-oxidants[43,44]. Nevertheless, major and minor constituents can participate in this antioxidant effect and not just one or a few minority active molecules[43].

α-glucosidase is a major enzyme in oligosaccharide hydrolysis. The α-glucosidase inhibition is an important approach to manage level of blood glucose. However, the major disadvantage of using inhibitors of α-glucosidase is their side effects such as diarrhea, distention of abdominal and flatulence[45]. Thus, it is important to evaluate antidiabetic activities of medicinal plants to develop alternative substances without side effects for diabetes mellitus and with low toxicity. The α-glucosidase inhibition activity of *C. trifurcatum* was evaluated for the first time. In this case, we can refer to some concepts such as synergism, antagonism and additivity between the chemical compounds to explain this inhibitory response for *Chrysanthemum trifurcatum*. A limited number of studies have evaluated α-glucosidase inhibition effect of the genus *Chrysanthemum*. Thus, n-butanol extract of *C. fuscum* may prevent hyperglycemia and hyperlipidemia in induced diabetic rats, which can be due to various mechanisms. This extract contained active substances which they produced a reduction of diabetic blood glucose levels[46]. Thi Luyen et al. demonstrated that the flavonones are the main compounds in *C. morifolium* flowers and showed a strong inhibition against α-glucosidase[47]. Therefore, these compounds may be important contributors to the hyperglycemia lowering property of this specie. The antidiabetic potential of essential oil can be due to the main constituents. Moreover, terpene constituents such as α-pinene inhibited the important enzymes related to diabetes type 2 essentially α-glucosidase[48]. However, the oil is a complex mixture of various molecules and a synergy between all molecules or the main molecules is responsible for its biological activities.

According to our knowledge, this is the first work on α-glucosidase and antioxidant activities and global chemical composition of organic extracts of different organs and aerial parts oil of *C. trifurcatum* from Tunisia. The obtained results showed that the aerial parts oil was rich in limonene and the content of phenolic compounds in the extracts was highly dependent on the organ of the plant. The highest level in polyphenol and flavonoid contents is detected in the leaves which favors their use in industry for the extraction of phenolic compounds. The inhibition of α-glucosidase and antioxidant activities of Tunisian *C. trifurcatum* may be due to the richness of this plant in phenolic compounds. So, this study should be followed by isolation, purification and identification of the specific molecules with high activity, from this plant, to develop natural α-glucosidase inhibitors and antioxidants.

**Conflict of interest statement**

The authors declare that they have no conflict of interest.

**References**

[1] Djeridane A, Yousfi M, Nadjeni B, Boutassouna D, Stocher P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem* 2006; 97(4): 654-660.

[2] Fang XK, Gao J, Zhu DN. Kaempferol and quercetin isolated from *Eucalyptus alatus* improve lucose uptake of 3T3-L1 cells without adipogenesis activity. *Life Sci* 2008; 82(11-12): 615-622.

[3] Scott LJ, Spencer CM. Miglitol: A review of its therapeutic potential in type 2 diabetes mellitus. *Drugs* 2000; 59(3): 521-549.

[4] Quezel P, Santa S. Nouvelle flore de l’Algérie et des régions désertiques méridionales. Tome II: Centre national de la recherche scientifique; 1981.

[5] Lee KD, Yang MS, Ha TJ, Park KM, Park KH. Isolation and identification of dihydrochrysosanolide and its 1-epimer from *Chrysanthemum coronarium* L. *Biosci Biotechnol Biochem* 2002; 66(4): 862-865.

[6] Pottier Alapetite G. La flore de la Tunisie: Angiospermes Dicotylédones Gamopétales. Tunisie: Publications scientifiques tunisiennes; 1981, p. 1000-1007.

[7] Le Floch E, Boulos L, Véla E, Ghrabi Gammar Z, Daoud Bouattour A, Ben Saad Limam S, et al. Flore de Tunisie, Catalogue synonymique commenté. Tunisie: Ministère de l’environnement et du développement durable et banque nationale de gènes; 2010, p. 101.

[8] Ben Sassi A, Harzallah-Skhir F, Chraief I, Bourougoun N, Hammami M, Aouni M. Chemical composition and antimicrobial activities of the essential oil of (Tunisian) *Chrysanthemum trifurcatum* (Desf.) Batt. and Trab. flower heads. *C R Chim* 2008; 11(3): 324-330.

[9] Ben Sassi A, Harzallah-Skhir F, Borgi W, Chouchène N, Aouni M. Effets de l’extrait méthanolique de *Chrysanthemum trifurcatum* (Desf.) Batt. et Trab. sur la motricité duodénale de rat. *C R Biol* 2007; 330(2): 226-300.

[10] Tahri W, Chatti A, Romero-Gonzalez R, López Gutiérrez N, Garrido Frenich A, Landoulsi A. A Phenolic profiling of the aerial part of *Chrysanthemum trifurcatum* using ultra high performance liquid chromatography coupled to Orbitrap high resolution mass spectrometry. *Anal Methods* 2016; 8(17): 3517-3527.

[11] Mokaddem-Daroui H, Toufek O, Kabouche A, Kabouche Z, Calliste CA, Durox JL. Components and antioxidant activity of the polar extracts of *Chrysanthemum trifurcatum*. *Chem Nat Compounds* 2012; 48(3): 498-499.

[12] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Enol Viticult* 1965; 16(3): 144-158.

[13] AOAC. *Official methods of analysis of the association of official analytical chemists*. 14th ed. Arlington: Ass Offic Anal Chem; 1984.

[14] Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem* 2004; 85(2): 231-237.

[15] Sun B, Richards-da-Silvia JM, Spranger I. Critical factors of vanillin assay for catechins and proanthocyanidins. *Agric Food Chem* 1998; 46(10): 4267-4274.

[16] Ben Sassi A, Harzallah-Skhir F, Chraief I, Bourougoun N, Hammami M, Aouni M. Essential oils and crude extracts from *Chrysanthemum trifurcatum* leaves, stems and roots: Chemical composition and antibacterial activity. *J Oleosci* 2014; 63(6): 607-617.

[17] Ramadan MF, Kroh LW, Morsel JT. Radical scavenging activity of black
cumin (Nigella sativa L.) coriander (Coriandrum sativum L.) and niger (Guizotia abyssinica Cass.) crude seed oils and oil fractions. J Agr Food Chem 2003; 51(24): 6961-6969.

[18]Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity apply ng an improved ABTS radical cation decolorization assay. Free Radical Biol Med 1999; 26(9-10): 1231-1237.

[19]Sanja SD, Sheth NR, Patel NK, Patel D, Patel B. Characterization and evaluation of antioxidant activity of Portulaca oleracea. Int J Pharm Pharm Sci 2009; 1(1): 74-84.

[20]Tao T, Zhang Y, Cheng Y, Wang Y. Rapid screening and identification of α-glucosidase inhibitors from mulberry leaves using enzyme-immobilized magnetic beads coupled with HPLC/MS and NMR. Biomed Chromatogr 2013; 27(2): 148-155.

[21]Davies NW. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. J Chromatogr A 1990; 503: 1-24.

[22]Adams R. Identification of essential oil components by gas chromatography/quadrapole mass spectroscopy. USA: Allured, Carol Stream IL; 2001, p. 19.

[23]Pedro LG, Santos PA, Silva JA, Figueiredo AC, Barroso JG, Deans SG. Essential oils from Azorean Laurus azorica. Phytochemistry 2001; 57(2): 245-250.

[24]Sibanda S, Chigwada G, Pool M, Gwebu ET, No-letto JA, Schmidt JM, Hamm S, Bleton J, Connan J, Tchapla A. A chemical investigation by gas chromatography/mass spectrometry of Thymus vulgaris L., sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. Ind Crops Prod 2013; 43: 827-831.

[25]Naaz M, Shahidi F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. J Pharm Biomed Anal 2006; 41(5): 1523-1542.

[26]Hudaib M, Speroni E, Di Pietra AM, Cavrini V. GC/MS evaluation of trilobatin from Heteropyxis trilobata. Phytochem Lett 2013; 6: 69-73.

[27]Jiyoung K, Jung NC, Kang MK, Daejung K, Jong SK, Jung HYP, et al. A correlation between antioxidant activity and metabolite release during the blanching of Chrysanthemum coronarium L. Bio Sci Biotechnol Biochem 2011; 75(4): 674-680.

[28]Trishna D, Hai LJ, Abul Hasnat M, Yunsuk K, Nadira BS, Pyo-Jam P, et al. Antioxidant potential and oxidative DNA damage preventive activity of Chrysanthemum indicum extracts. J Food Biochem 2013; 37(4): 440-448.

[29]Aneta TP, Dasha SM, Jordanka NA. The effect of freezing on the antioxidant activity of Bulgarian Chrysanthemum balsamita. J BioSci Biotech 2014; 3: 17-21.

[30]Amrani A, Zama D, Boubekri N, Ben aissa O, Meraihi Z, Benayache F, et al. The protective effect of Chrysanthemum fantanexii extract, vitamin E and C on sodium valproate-induced embryotoxicity in pregnant mice. J Med Plants Res 2012; 6(19): 3535-3544.

[31]Lu Y, Foo LY. Antioxidant activities of polyphenols from sage (Salvia officinalis). Food Sci 2001; 75(2): 197-202.

[32]Xie YY, Yuan D, Tian HF. Chemical constituents in the flowers of Chrysanthemum morifolium Ramat. Chin J Med Chem 2009; 19(4): 276-279.

[33]Liu JQ, Shen QQ, Liu JS, Wu DL, Wang JT. Studies on the chemical constituents from Chrysanthemum morifolium Ramat. China J Chin Mater Med 2001; 26(8): 547-548.

[34]Ha CL, Weng CY, Wang L, Lian TW, Wu MJ. Immunomodulatory effect of Glossogynce tenuifolia in murine peritoneal macrophages and splenocytes. J Ethnopharmacol 2006; 107(1): 116-125.

[35]Fallah H, Ksouri R, Oueslati S, Guyot S, Magné C, Abdelly C. Interspecific variability of antioxidant activities and phenolic composition in Mesembryanthemum genus. Food Chem Toxicol 2009; 47(9): 2308-2313.

[36]Ding M, Zhao JS, Bowman L, Lu YJ, Shi XL. Inhibition of AP-1 and MAPK signaling and activation of Nrf2/ARE pathway by quercitrin. Int J Oncol 2010; 36(1): 59-67.

[37]Bahramiakia S, Ardestani A, Yazdanparast R. Protective effects of four Iranian medicinal plants against free radical-mediated protein oxidation. Food Chem 2009; 115(1): 37-42.

[38]Zhang L, Ravipati AS, Koyyalamudi SR, Jeong SC, Reddy N, Smith PT, et al. Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. J Agric Food Chem 2011; 59(23): 12361-12367.

[39]Wang W, Wu N, Zu YG, Fu YJ. Antioxidative activity of Rosmarinus officinalis L. essential oil compared to its main components. Food Chem 2008; 108(3): 1019-1022.

[40]Marostica MR, Taare S, Franch GC, Nowill A, Pastore GM, Hyslop S. Antioxidant potential of arome compounds obtained by limonene biotransformation of orange essential oil. Food Chem 2009; 116(1): 8-12.

[41]Dong HQ, Li M, Zhu F, Liu FL, Huang JB. Inhibitory potential of omega-3 fatty acids and fenugreek essential oil key enzymes of carbohydrate-digestion and hypertension in diabetes rats. Lipids Health Dis 2011; 10: 226.