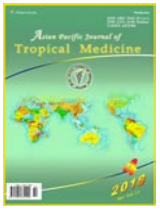

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Antioxidant and α-glucosidase activities and phytochemical constituents of *Chrysanthoglossum trifurcatum* (Desf.)

Ahlem Ben Sassi[1]✉, Sana Amroussi[1], Malek Besbes[1], Mahjoub Aouni[1], Fethia Skhiri[2]

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

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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 (IC_{50}=0.68 mg/mL) and flowers (IC_{50}=0.67 mg/mL) methanolic extracts and essential oil (IC_{50}=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 IC_{50} 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.

✉First and corresponding author: Dr. Ahlem Ben Sassi, Laboratory of Transmissible Diseases and Biologically Active Substances, Department of Microbiology, Faculty of Pharmacy, Avicenne street, Monastir, Tunisia.
Tel: + 216 98 94 74 26
Fax: + 216 73 46 57 54
E-mail: ahlem_ben_sassi@yahoo.fr

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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 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 of *C. trifurcatum*[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_2SO_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, 1×10^4 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:

% inhibition of radical DPPH=$[(A_{control} - A_{sample})/A_{control}]\times100$.

Where, $A_{control}$ is control reaction absorbance, and A_{sample} is sample test absorbance. Thereafter, IC_{50} 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 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 following formula:

% inhibition ratio=$[(A_{control} - A_{sample})/A_{control}]\times100$.

Where, $A_{control}$ is control reaction absorbance, and A_{sample} is sample test absorbance. IC_{50} 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 ferric cyanide [1% (w/v), 2.5 mL]. Then, trichloroacetic acid [10% (w/v), 2.5 mL) was added after 20 min at 50 ℃ A centrifugation at 3 000 r/min for 10 min was made. The supernatant (2.5 mL), distilled water (2.5 mL) and ferric chloride (0.1%, 1 mL) were mixed. After 10 min at ambient temperature, absorbance was measured at 700 nm. Trolox is used as a reference product. The reducing power was determined by the formula:

% reducing power=$[(A_{sample}/A_{control} -1)]$ 100.

Where, A_{sample} is sample absorbance and $A_{control}$ is control absorbance.

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 ℃ for 15 min. Acarbose was a standard. Absorbance was measured at 405 nm. Inhibition ratio (%) by samples and acarbose were determined by the equation:

% inhibition ratio=$[1-(A_{sample}/A_{control})]\times100$.

Where, A_{sample} is sample absorbance and $A_{control}$ 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 IC_{50} (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 extract (Table 1). The methanolic extract of leaves had the highest contents of total phenol, flavonoid, flavonol and tannin. However, petroleum ether stem extract presented lower level of polyphenols

Table 1

Total polyphenol, flavonoid, flavonol and tannin contents in *C. trifurcatum* according to organs and solvent extraction systems.

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 ± 0.45^{b}	92.84 ± 8.78^{d}	15.34 ± 0.65^{c}	5.39 ± 0.10^{d}
	Ethyl acetate	0.60	1.59 ± 0.33^{b}	46.62 ± 1.05^{b}	5.51 ± 1.46^{a}	0.05 ± 0.002^{a}
	Methanol	5.10	20.85 ± 2.68^{i}	634.81 ± 1.39^{j}	129.95 ± 1.07^{e}	2.12 ± 0.06^{c}
Leaves	Petroleum ether	2.92	12.56 ± 2.96^{h}	522.43 ± 2.86^{i}	357.32 ± 1.66^{f}	4.87 ± 0.30^{d}
	Ethyl acetate	1.69	40.59 ± 4.08^{j}	$3\ 278.37\pm10.41^{k}$	505.99 ± 1.05^{g}	4.67 ± 0.20^{d}
	Methanol	6.20	142.51 ± 1.29^{k}	$4\ 625.83\pm7.89^{l}$	624.13 ± 1.18^{h}	11.92 ± 0.20^{e}
Stems	Petroleum ether	0.29	0.27 ± 0.02^{a}	13.44 ± 3.55^{a}	12.77 ± 1.18^{bc}	0.34 ± 0.03^{b}
	Ethyl acetate	0.50	1.69 ± 0.37^{c}	151.07 ± 1.20^{h}	16.92 ± 1.88^{c}	0.44 ± 0.01^{b}
	Methanol	1.10	3.20 ± 0.46^{d}	95.27 ± 1.32^{e}	61.35 ± 1.85^{d}	1.15 ± 0.04^{c}
Roots	Petroleum ether	0.42	6.18 ± 0.68^{f}	107.46 ± 2.50^{g}	10.73 ± 0.62^{b}	0.31 ± 0.01^{b}
	Ethyl acetate	0.52	5.45 ± 0.20^{e}	57.52 ± 2.04^{c}	8.24 ± 0.61^{ab}	0.30 ± 0.01^{b}
	Methanol	0.91	7.86 ± 0.53^{g}	99.76 ± 2.95^{f}	65.92 ± 0.35^{d}	0.42 ± 0.05^{b}

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-terpinene (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 IC_{50}, 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 extracts from flowers and leaves. Also, these activities were lower than a Trolox with IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} of 0.044, 0.045 and 0.049 mg/mL, respectively. Ethyl acetate extracts of stems (IC_{50}=0.054 mg/mL) and flowers (IC_{50}=0.057 mg/mL) also exhibited α-glucosidase inhibition, as well as methanolic stem extract (IC_{50}=0.064 mg/mL). This biological activity was better than that of acarbose (IC_{50}=0.07 mg/mL).

Table 2

Constituents of the essential oil from the aerial parts of *C. trifurcatum* (Desf.).

Compound[a]	RI[b]	Content (%)[c]	Identification
2-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
Sabinene	976	1.21	GC–MS, RI
β-Pinene	979	2.71	GC–MS, RI
β-Myrcene	991	0.76	GC–MS, RI
Limonene	1 032	29.21	GC–MS, RI
γ-Terpinene	1 064	12.96	GC–MS, RI
α-Terpinolene	1 089	1.02	GC–MS, RI
1-Octen-3yl-acetate	1 096	trd	GC–MS, RI
Camphor	1 145	0.36	GC–MS, RI
Borneol	1 169	0.24	GC–MS, RI
p–Cymen-8-ol	1 187	0.79	GC–MS, RI
α-Terpineol	1 191	0.78	GC–MS, RI
Myrtenal	1 193	0.81	GC–MS, RI
Myrtenol	1 196	1.72	GC–MS, RI
Fenchyl acetate	1 225	0.46	GC–MS, RI
Carvacrol	1 291	1.06	GC–MS, RI
4-Terpenyl acetate	1 340	12.18	GCMS, RI
α-Cubebene	1 352	1.14	GC–MS, RI
α-Terpenyl acetate	1 354	3.68	GC–MS, RI
α-Ylangene	1 372	trd	GC–MS, RI
α-Copaene	1 379	1.24	GC–MS, RI
β-Bourbobene	1 381	1.13	GC–MS, RI
α-Gurjunene	1 409	2.05	GC–MS, RI
β-Caryophyllene	1 420	0.18	GC–MS, RI
β-Gurjunene	1 434	0.25	GC–MS, RI
α-Cedrene	1 437	1.07	GC–MS, RI
α-Himachalene	1 447	1.24	GC–MS, RI
β-Farnesene	1 458	1.11	GC–MS, RI
Germacrene-D	1 484	0.19	GC–MS, RI
β-Selinene	1 489	0.26	GC–MS, RI
α-Muurolene	1 502	trd	GC–MS, RI
β-Bisabolene	1 508	trd	GC–MS, RI
δ-Cadinene	1 523	1.09	GC–MS, RI
Elemol	1 549	0.53	GC–MS, RI
Germacrene-B	1 555	0.18	GC–MS, RI
β-Calacorene	1 561	0.94	GC–MS, RI
β-Spathulenol	1 576	0.16	GC–MS, RI
τ-Cadinol	1 640	2.29	GC–MS, RI
τ-Muurolol	1 642	0.12	GC–MS, RI
α-Cadinol	1 654	trd	GC–MS, RI
Total identified		98.95	
Yield (%)(w/w)		0.06	

GC: Gas chromatography; MS: Mass spectrometry; RI: Retention indices. [a]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]. [b]RI relatives to *n*-alkanes (C9-C28) on the apolar HP-5MS column. [c]The contents (%) were calculated by electronic integration of the FID peak areas obtained on the apolar HP-5MS column; trd: Trace (<0.05%);.

Table 3

Antioxidant capacity determined by DPPH, ABTS and reducing power test systems of *C. trifurcatum* extracts and oil.

Organ	Solvent extraction	DPPH radical*		ABTS radical*		Reducing power*	
		Scavenging activity at 3.00 mg/mL (%)	IC_{50} (mg/mL)**	Scavenging activity at 3.00 mg/mL (%)	IC_{50} (mg/mL)**	Reducing power at 3.00 mg/mL (%)	EC_{50} (mg/mL)***
Flowers	Petroleum ether	23.95±1.20^{a}	>3.00	34.50±3.20^{b}	>3.00	51.55±0.98^{c}	2.89±0.04
	Ethyl acetate	67.05±1.51^{c}	1.10±0.02	44.88±1.10^{a}	>3.00	53.94±2.88^{b}	2.79±0.02
	Methanol	77.74±1.80^{b}	0.67±0.02	70.17±3.11^{a}	1.79±0.01	79.86±0.68^{b}	1.54±0.05
Leaves	Petroleum ether	28.64±1.31^{a}	>3.00	38.26±3.71^{b}	>3.00	54.62±0.95^{c}	2.72±0.11
	Ethyl acetate	38.97±1.21^{b}	>3.00	27.43±2.60^{a}	>3.00	64.52±0.90^{c}	2.10±0.05
	Methanol	69.00±1.01^{b}	1.45±0.05	51.94±3.25^{a}	2.68±0.05	77.14±0.01^{b}	1.61±0.03
Stems	Petroleum ether	23.35±1.11^{a}	>3.00	81.79±1.90^{c}	2.11±0.03	56.62±0.76^{b}	2.66±0.12
	Ethyl acetate	85.46±1.25^{b}	1.76±0.03	82.19±2.22^{b}	1.96±0.02	50.77±0.49^{a}	2.93±0.03
	Methanol	90.32±1.50^{c}	0.68±0.01	82.65±2.90^{b}	1.32±0.07	58.08±0.77^{a}	2.52±0.04
Roots	Petroleum ether	31.56±1.72^{a}	>3.00	32.18±2.72^{a}	>3.00	50.53±0.51^{b}	2.96±0.02
	Ethyl acetate	46.94±1.05^{a}	>3.00	39.18±1.40^{a}	>3.00	45.07±0.39^{a}	>3.00
	Methanol	53.50±1.20^{b}	1.21±0.05	38.54±1.55^{a}	>3.00	51.20±1.06^{b}	2.90±0.02
Aerial parts	Essential oil	82.56±3.20^{a}	0.72±0.01	73.92±1.50^{a}	0.87±0.03	85.32±1.43^{a}	0.92±0.09
	Trolox	99.89±0.04^{a}	0.14±0.02	99.49±0.05^{a}	0.16±0.01	99.78±0.10^{a}	0.08±0.02

*Each value is expressed as mean±standard deviation (n=6). Means in each line followed by different letters are significantly different (P<0.05). **IC_{50} inhibitory concentration at which 50% of radicals are inhibited. ***EC_{50} effective concentration at 50%.

Table 4

α -glucosidase inhibition of *C. trifurcatum* extracts and oil.

Organ	Solvent extraction	IC_{50} (mg/mL)
Flowers	Petroleum ether	0.088±0.001^{j}
	Ethyl acetate	0.057±0.002^{d}
	Methanol	0.092±0.001^{l}
Leaves	Petroleum ether	0.044±0.001^{a}
	Ethyl acetate	0.074±0.001^{g}
	Methanol	0.086±0.002^{i}
Stems	Petroleum ether	0.090±0.002^{k}
	Ethyl acetate	0.054±0.002^{c}
	Methanol	0.064±0.002^{e}
Roots	Petroleum ether	0.045±0.001^{a}
	Ethyl acetate	0.092±0.002^{l}
	Methanol	0.083±0.002^{h}
Aerial parts	Essential oil	0.049±0.001^{b}
Acarbose		0.070±0.001^{f}

IC_{50} values are shown as mean±SD of three independent experiments. Different letters show significant differences (P<0.05).

4. Discussion

For phenolic composition, results demonstrated that methanol was better solvent in extracting polyphenols. Therefore, the polarity of solvent will play an important role in increasing solubility of phenols[26,27]. Then, variation of phenol accumulation between plant parts should be related to their specific cells and tissues. The high presence of phenols in leaves is attributed to the phenological organ growth, transport involved in polyphenols distribution at plant level, handy interaction between plant parts and processes of degradation and/or biosynthesis[28].

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]. Isorhamnetin was identified in flowers, leaves and stems of *C. trifurcatum*. In contrast, flavones, flavonols and phenolic acid (caffeic 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 (caffeic 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* aerial parts collected in Algeria exhibited antioxidant activity by DPPH test with IC_{50}=0.199 mg/mL[11]. Others findings reported a potent antioxidant activity of *Chrysanthemum* genus such *Chrysanthemum morifolium*[30], *Chrysanthemum coronarium*[31], *Chrysanthemum indicum*[32], *Chrysanthemum balsamita*[33] and *Chrysanthemum fuscatum*[34]. However, Tahri *et al*. reported that methanol extracts of *C. trifurcatum* were rich in flavonoidslike luteolin and phenolic acid like caffeic acid[10]. It is shown that the caffeic acid is a potent radical scavenger and it is more effective than Trolox[35]. Luteolin is an important component in *Chrysanthemum* species[36]. *Chrysanthemum morifolium* contained luteolin-7-*O*- β -*D*-glucoside which had antioxidant and inflammatory activities[37-39]. *Chrysanthemum indicum* is considered as an important source of quercitrin and myricetin. Others researchers have reported that quercetin had antioxidant activity[39,40]. Bahramikia *et al*. indicated that flavonoids interrupt free radical autoxidation chain propagation[41]. According to Zhang *et al*.[42], phenolic and flavonoid plant compounds generally contribute to antioxidant activity. So,

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. fuscatum* 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 flavones 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.

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