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Original Article

Diplotaxis simplex suppresses postprandial hyperglycemia in mice by inhibiting key-enzymes linked to type 2 diabetes

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ABSTRACT

Nutritional properties of *Diplotaxis simplex* Spreng., Brassicaceae, an edible wild cruciferous largely distributed in North Africa, were investigated. Potassium (3690–3780 mg/100 g) and calcium (900–1170 mg/100 g) were the most concentrated minerals. Linoleinic acid was found to be the main fatty acid (25.4–27.7%), followed by palmitic acid (13.2–15.3%). Moreover, lipidic fraction of leaves was characterized by a relatively high rate of ethyl linoleate (14.4%) and phytol (17.6%). Ethyl acetate extract of *D. simplex* flowers showed concentration-dependent α-amylase (IC_{50} 3.46 mg/ml) and α-glucosidase (IC_{50} 0.046 mg/ml) inhibitory activities. The positive *in vitro* enzymes inhibition was confirmed by a maltose tolerance test, which showed that treatment with flowers extract significantly inhibited the rise in blood glucose levels of maltose-loaded mice comparable to the standard antihyperglycemic agent acarbose. From these results, it may be concluded that *D. simplex* flowers can be used effectively as a safer alternative therapy to control postprandial hyperglycemia.

Introduction

Diabetes mellitus (DM) is a chronic disease characterized by a deficiency in insulin production and its action or both. Consequently, it leads to elevated blood glucose levels with disturbances in most metabolic processes. In recent years DM has become a major health problem worldwide, reaching epidemic proportion. In fact, DM affects more than 200 million people worldwide and it is expected to reach 300 million by 2025 (Singab et al., 2014). Hyperglycemia is considered as a main cause of complications related to coronary artery disease, cardiovascular disease, renal failure, blindness, neurological complications and premature death. Therefore, control of postprandial blood glucose level is critical in the early treatment of DM and in reducing chronic vascular complications (Lopez-Candales, 2001). The glucosidase inhibitors such as acarbose and miglitol inhibit enzymes responsible for the breakdown of carbohydrates in the small intestine. They act mainly by decreasing the glucose absorption level and consequently, they display antihyperglycemic effects (Ross et al., 2004). Synthetic hypoglycemic agents produce serious side effects, whereas bioactive compounds derived from natural resources are frequently considered safe and cost effective (Rao and Jamil, 2011). Thus, plants may play an important role in drug development programs.

Medicinal, aromatic, and culinary plants have always been part of human life, as they were used for food and medicine. Nevertheless, for long period these plants have been forgotten or neglected in our food and that can be exploited for their many virtues. Currently, several studies focus on phytochemical characterization and evaluation of biological properties of various plants. In fact, many extracts or compounds from plants have shown interesting pharmacological properties and they have become very popular as potential agents for natural health and/or human nutrition. Thereby, the plant kingdom is a very promising and probably inexhaustible source of drugs, nutraceuticals and food ingredients. However, very few plants have been well studied and a large majority expects to be interested. *Diplotaxis simplex* Spreng. (Vernacular name: Jarjir) is a wild edible plant from the Brassicaceae family, which represents an important herb species in Tunisia. It is an annual plant, glabrous and stems are much branched, which can reach 50 cm of height. The leaves form a basal rosette and the bright yellow flowers appear in winter to late spring.

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This plant grows in many sandy, loamy and stony soils in coast and south of the country (Chaieb and Boukhris, 1998). Many previous studies refer to the genus *Diplotaxis* as traditionally used plants with therapeutic properties. Moreover, several species of *Diplotaxis* are reported as food crops in different regions, and may contribute to differentiation in the fresh food supply chain. In fact, these herbs are appreciated for their strong pungent flavor and they are consumed raw or cooked, in salads and soups (Guarrera, 2003; Mohammed et al., 2013).

Few studies investigated the nutritional and biological properties of *D. simplex*. Consequently, the objective of the present work is to investigate the mineral composition of its leaves and flowers. Analysis of lipid compounds by GC–FID and GC–MS techniques was also carried out. Moreover, the inhibitory effects of flowers extract on α-amylase and α-glucosidase activities as well as the postprandial hyperglycemia tests were also studied.

Material and methods

Plant material

Diplotaxis simplex Spreng., Brassicaceae (Fig. 1) was collected from south-eastern of Tunisia (Medenine, with an arid climate characterized by a mean rainfall of 150 mm/year). The plant specimens were identified by Pr. Mohamed Debouba, botanist in the High Institute of Applied Biology of Medenine (Medenine, Tunisia), where voucher specimens [Ds01] have been deposited. After harvest, leaves and flowers were separated and dried in the shadow, until constancy of the mass (20 days), then ground into fine powder and stored at ambient temperature in a dry place and in the dark until use.

Animals

Male albino mice with body masses of 20–25 g, obtained from the Veterinary Research Institute (Sfax, Tunisia), were used in this study. The animals were maintained under standard environmental conditions of temperature, relative humidity and a 12 h dark/light cycle. They had *ad libitum* access to food and water. The experimental protocol was performed according to the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe No. 123, Strasbourg, 1985). Approval for these experiments was obtained from the Medical Ethics Committee for the Care and Use of Laboratory Animals of the Pasteur Institute of Tunis (approval number: FST/LNFP/Pro 152012).

Chemical composition and mineral concentrations

Moisture, ash, carbohydrate and total protein were determined according to A.O.A.C. (1995). The fat content was determined by Soxhlet extraction with hexane for 8 h at boiling point of the solvent. Different mineral constituents (potassium [K], calcium [Ca], sodium [Na], magnesium [Mg], iron [Fe] and copper [Cu]) were analyzed separately using an atomic absorption spectrophotometer (Hitachi Z6100, Tokyo, Japan). The lipid compounds from leaves and flowers were extracted using chloroform/methanol as previously described by Zouari et al. (2010). After that, methyl esters of the fatty acids were prepared and analyzed by Gas Chromatography–Flame Ionization Detector (GC–FID) and Gas Chromatography–Mass Spectrometry (GC–MS) techniques.

Lipid compounds analyses

Gas Chromatography–Flame Ionization Detector (GC–FID)

An Agilent Technologies 6890N gas chromatograph equipped with HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Hewlett-Packard) and connected to a flame ionization detector (FID) was used. The column temperature was programmed at 50 °C for 1 min, then 7 °C/min to 250 °C, and then left at 250 °C for 5 min. The injection port temperature was 240 °C; while of the detector was 250 °C (split ratio: 1/60). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min and the analyzed sample volume was 2 μl. Percentages of the compounds was calculated by electronic integration of FID peak areas, without the use of response factor correction. Retention indices (RI) were calculated for separate compounds relative to C_7–C_{25} n-alkanes mixture (Aldrich Library of Chemicals Standards, Saint-Louis, Missouri, USA) (Kovàts, 1958).

Gas Chromatography–Mass Spectrometry (GC–MS)

The lipid compounds were also analyzed by GC–MS, using an Agilent Technologies 6890N gas chromatograph. The fused HP-5MS capillary column (the same as that used in the GC–FID analysis) was coupled to an Agilent Technologies 5973B mass-spectrometer (Hewlett-Packard, Palo Alto, CA, USA). The oven temperature was programmed at 50 °C for 1 min, then 7 °C/min to 250 °C, and then left at 250 °C for 5 min. The injection port temperature was 250 °C and that of the detector was 280 °C (split ratio: 1/100). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min. The mass spectrometer conditions were as follows: ionization voltage, 70 eV; ion source temperature, 150 °C; electron ionization mass spectra were acquired over the mass range 50–550 *m*/*z*.

Lipid compounds identification

The lipid compounds were identified by comparing the mass spectra data with spectra available from the Wiley 275 mass spectra libraries (software, D.03.00). Further identification confirmations were made referring to retention indices data generated from a series of known standards of *n*-alkanes mixture (C_7–C_{25}) (Kovàts, 1958) and to those previously reported in the literature (Adams, 2007).

Preparation of D. simplex extracts

The dried powder of the *D. simplex* leaves or flowers (25 g) was Soxhlet-extracted with 300 ml ethyl acetate during 6 h. The solvent was then evaporated using a rotary evaporator and the residual solvent was removed by flushing with nitrogen. Finally, the obtained extracts were kept in the dark at 4 °C until further analysis.

Biochemical assays for determining the inhibition of enzymes activities

α-Amylase inhibition assay

The α-amylase inhibition assay was performed according to the method described by Deguchi et al. (2003) with slight modifications. Briefly, the assay mixture consisted of 500 μl of 1% starch solution, 400 μl of 0.1 M sodium phosphate buffer (pH 7.0), 50 μl of plant extract dissolved in dimethyl sulfoxide (DMSO) and 50 μl of pancreatic α-amylase (Sigma, St. Louis, USA) solution (2 U/ml). Then, the reaction medium was incubated at 37 °C for 10 min followed by addition of 3 ml of 3,5-dinitrosalicylic acid (DNS) color reagent. Finally, the solution was placed in a boiling water bath for 5 min, diluted with 20 ml of distilled water and the absorbance was measured at 540 nm. Absorbance of a control sample was prepared accordingly without plant extract and acted as negative control. The standard antihyperglycemic agent acarbose was used as positive control. The experimental extract and acarbose were tested

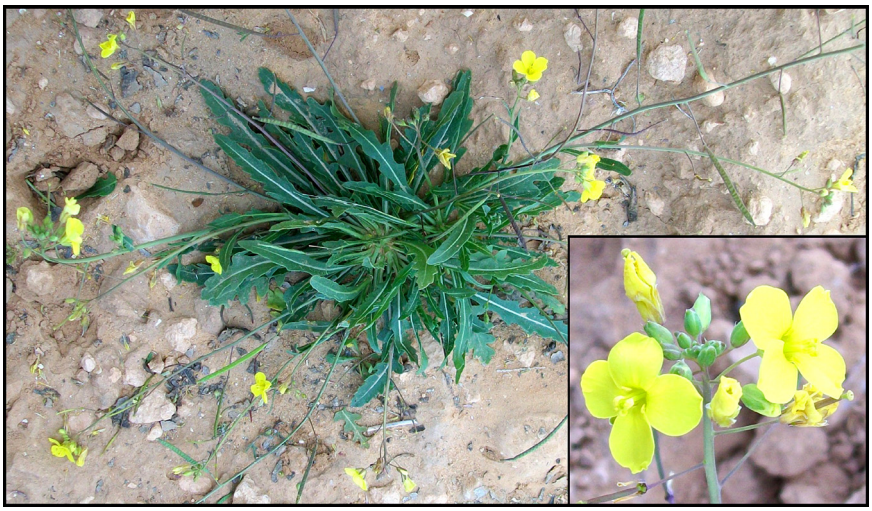

Fig. 1. *Diplotaxis simplex* Spreng., Brassicaceae, collected from south-eastern Tunisia (Medenine: Boughrara area at 19 m altitude, latitude 33°30′91″ N, longitude 10°38′26″ E).

with varying concentrations from 1.25 to 5 mg/ml. The results were expressed as percentage inhibition using the following formula:

$$\text{Inhibition}\,(\%) = \left[\frac{\text{Ac} - \text{As}}{\text{Ac}}\right] \times 100$$

where Ac is the absorbance of the control reaction without sample and As is the absorbance of the sample. IC_{50} value, defined as the sample concentration (mg/ml) at which 50% inhibition of the enzyme activity occurs, was calculated from the graph plotting enzyme inhibition against sample concentration. All tests were carried out for three sample replications and the results were averaged.

α-Glucosidase inhibition assay

Initially, mice were dissected and small intestines tissues were used to prepare a crude extract as a source of intestinal α-glucosidase. Small intestines were excised from ten mice and suspended in 20 ml of 0.1 M potassium phosphate buffer (pH 7.0) containing 5 mM ethylenediaminetetraacetic acid (EDTA). The suspension was homogenized for 15 min and after vigorous stirring for 1 h, the suspension was centrifuged. The supernatant was dialyzed against 0.01 M potassium phosphate buffer (pH 7.0) for 24 h. Finally, the enzyme solution was lyophilized and stored at 4 °C until further use. Then, the α-glucosidase inhibitory activity was measured as described by Matsui et al. (2001) with slight modifications. Briefly, the enzymatic reaction was performed using *p*-nitrophenyl-α-D-glucopyranoside (pNPG) in 0.1 M phosphate buffer (pH 6.8) as substrate. All reactions were carried out at 37 °C for 30 min. The enzymatic activity was quantified by measuring p-nitrophenol released from pNPG at a wavelength of 400 nm and compared to a control which had DMSO solution in place of the plant extract. The experimental extract and the standard acarbose were tested with varying concentrations from 0.02 to 0.5 mg/ml. The α-glucosidase inhibitory activity was expressed as percentage inhibition using the following formula:

$$\text{Inhibition}\,(\%) = \left[\frac{\text{Ac} - \text{As}}{\text{Ac}}\right] \times 100$$

where Ac is the absorbance of the control reaction without sample and As is the absorbance of the sample. The 50% inhibition concentration (IC_{50}, mg/ml) of plant extract against intestinal α-glucosidase was calculated. All tests were carried out for three sample replications and the results were averaged.

In vivo *maltose and glucose tolerance tests*

The experimental animals were classified into six groups, each of them with three mice ($n = 3$). Three groups were used for maltose tolerance test and others for glucose tolerance test. For the first administration by oral gavage, mice were treated with 200 mg/kg of body mass of flowers extract (test group) or 200 mg/kg of body mass of antihyperglycemic agent acarbose (positive control group). Mice of the negative control group received physiological NaCl-solution. One hour later, either maltose or glucose solution (3 g/kg of body mass) was loaded for the mice as the second administration. Blood samples were collected from the tail vein before oral administration and at 30, 60 and 90 min thereafter, according to procedures outlined in the Institutional Animal Care and Use Committee Guideline (1999). Blood glucose levels were determined using a glucometer with its corresponding glucose-test strips (ACCU-CHEK Meter®, Roche Diagnostics Corp., Kalamazoo, USA).

Statistical analysis

All analytical determinations were performed at least in triplicate. Values were expressed as the mean ± standard deviation. Analysis of variance was conducted, and differences between variables were tested for significance by one-way analysis of variance using SPSS 11 (Statistical Package for the Social Sciences, The Predictive Analytics Company, Chicago IL, USA). A difference was considered statistically significant at least when $p < 0.05$.

Results and discussion

Leaf and flower chemical compositions

The results of the nutrient composition (protein, carbohydrate, fat and ash) expressed on a dry mass basis were presented in Table 1. Carbohydrates (47.97–58.14 g/100 g) followed by proteins (22.86–26.52 g/100 g) were the most abundant macronutrients for both leaves and flowers. The protein content of *D. simplex* leaves (22.86 g/100 g) was found to be much higher than the value reported for *Malva aegyptiaca* leaves (8.7 g/100 g), which is a wild edible vegetable (Zouari et al., 2011). However, protein contents of *D. simplex* remain lower than those of other edible leafy vegetables, in which protein contents ranged from 30.0 to 34.6 g/100 g dry mass (Aletor et al., 2002). The ash content varied between 11.14 g/100 g in flowers and 26.92 g/100 g in leaves. Concentrations

Table 1
Moisture (g/100 g fresh mass) and macronutrient composition (g/100 g dry mass) of *D. simplex* leaves and flowers.

	Moisture	Ash	Proteins	Fat	Carbohydrates
Leaves	69.26 ± 0.65[a]	26.92 ± 0.96[a]	22.86 ± 0.46[a]	2.25 ± 0.36[a]	47.97 ± 1.78[a]
Flowers	67.21 ± 0.70[a]	11.14 ± 0.86[b]	26.52 ± 0.55[a]	4.20 ± 0.40[b]	58.14 ± 1.81[b]

Data presented as the mean ± standard deviation ($n = 3$). Different lowercase superscript letters indicate significant differences ($p < 0.05$).

Table 2
Mineral concentrations (mg/100 g dry matter) in *D. simplex* leaves and flowers.

	Leaves	Flowers
K	3780 ± 45[a]	3690 ± 65[a]
Ca	1170 ± 18[a]	900 ± 11[b]
Na	100 ± 20[a]	770 ± 12[b]
Mg	320 ± 7[a]	510 ± 8[b]
Fe	1.7 ± 0.03[a]	60.1 ± 0.14[b]
Cu	<0.50	<0.50

Data presented as the mean ± standard deviation ($n = 3$). Different lowercase superscript letters indicate significant differences ($p < 0.05$).

of different minerals (K, Ca, Mg, Na, Fe and Cu) in leaves and flowers were presented in Table 2. Potassium (3690–3780 mg/100 g) and calcium (900–1170 mg/100 g) were the most concentrated minerals, followed by sodium (100–770 mg/100 g) and magnesium (320–510 mg/100 g). Mineral contents (K, Ca and Mg) of *D. simplex* were comparable with those reported for various edible wild plants species (Guil et al., 1998) and much higher than those of some leafy vegetables (Aletor et al., 2002). It seems that *D. simplex*, growing wild in arid and semi-arid regions, contained sufficient amounts of macro-minerals that satisfy human requirements.

Fat was the less abundant macronutrient and its content was found to be comparable with that reported for various edible leafy vegetables (Aletor et al., 2002; Zouari et al., 2011). Compositions of the lipidic fractions extracted from *D. simplex* leaves and flowers were investigated using both GC–FID and GC–MS techniques. The percentages and the retention indices of the identified compounds are listed in Table 3 in the order of their elution on the HP-5MS column. The global chromatographic analysis resulted in the identification of 16 compounds, accounting for 83.5% and 85.5% of the total lipid content of flowers and leaves, respectively. Leaves and flowers of *D. simplex* presented comparable fatty acid profiles. Linoleinic acid (C18:3 n−3) was found to be the main fatty acid (25.4–27.7%), followed by palmitic acid (C16:0) (13.2–15.3%) for both leaves and flowers. Linolenic acid, linoleic acid and palmitic acid were also found to be the major fatty acids of the edible wild *M. aegyptiaca* (Zouari et al., 2011). Furthermore, lipidic fraction extracted from *D. simplex* flowers contained some hydrocarbons such as hexacosane (5.7%) and pentacosane (5.1%), which were absent in lipidic fraction of leaves. However, leaves' lipidic fraction was characterized by a relatively high rate of ethyl linoleate (14.4%) and phytol (17.6%), which were detected at low concentrations in flowers (Table 3). Recently, Santos et al. (2013) reported that phytol, an acyclic monounsaturated diterpene alcohol from chlorophyll, presented pronounced antinociceptive activity in mice.

In vitro *inhibitory effect on α-amylase and α-glucosidase activities*

α-Amylase and α-glucosidase play major roles in carbohydrate hydrolysis and absorption. The inhibition of these enzymes would delay the degradation of the complex sugars such as starch and prolong overall carbohydrate digestion time, which prevent an excessive postprandial blood glucose rise. Therefore, controlling glucose production from food sources using an oral α-amylase and α-glucosidase inhibitor would be an effective management for non-insulin-dependent DM patients (Ross et al., 2004).

Ethyl acetate extract of *D. simplex* flowers was tested for the inhibition assays of α-amylase (Fig. 2A) and α-glucosidase (Fig. 2B), using acarbose as a positive control. α-Amylase and α-glucosidase activities decreased with the concentration enhancement of the plant extract or acarbose, which shows a dose-dependent response. Fig. 2A showed that α-amylase inhibition rate by the plant extract was lower than that obtained by acarbose. α-Glucosidase inhibitory activity was measured at 0.02–0.5 mg/ml of plant extract

Table 3
Mean percentage of lipid compounds extracted from *D. simplex* leaves and flowers.

	Compounds	Concentration (%)[a]		RI[b]
		Leaves	Flowers	
1	Octane	–	2.6	796
2	Nonane	2.8	1.0	895
3	Octanoic acid, methyl ester (C8:0)	–	1.0	1117
4	Decanoic acid, methyl ester (C10:0)	–	0.9	1313
5	Dodecanoic acid, methyl ester (C12:0)	–	1.0	1511
6	Tetradecanoic acid, methyl ester (C14:0)	–	3.4	1708
7	*Ethyl linoleate*[c]	*14.4*	1.4	1876
8	*Hexadecanoic acid, methyl ester (C16:0)*[c]	*13.2*	*15.3*	1899
9	Heptadecanoic acid, methyl ester (C17:0)	–	0.5	1961
10	9,12-Octadecadienoic acid, methyl ester (C18:2 n−6)	4.4	5.6	2071
11	*9,12,15-Octadecatrienoic acid, methyl ester (C18:3 n−3)*[c]	*25.4*	*27.7*	2081
12	*Phytol*[c]	*17.6*	3.7	2097
13	Octadecanoic acid, methyl ester (C18:1)	7.7	6.1	2110
14	Eicosanoic acid, methyl ester (C20:0)	–	2.5	2312
15	Pentacosane	–	5.1	2510
16	Hexacosane	–	5.7	2586
	Total	85.5	83.5	

Lipid fractions were methylated before analysis by GC–FID and GC–MS techniques. Results are mean values of three samples ($n = 3$). Compounds are listed in order of their elution from a HP-5MS column.

[a] Percentages obtained by FID peak-area normalization.

[b] RI calculated against C_7–C_{25} *n*-alkane mixture on the HP-5MS column.

[c] Main compounds are in italics font.

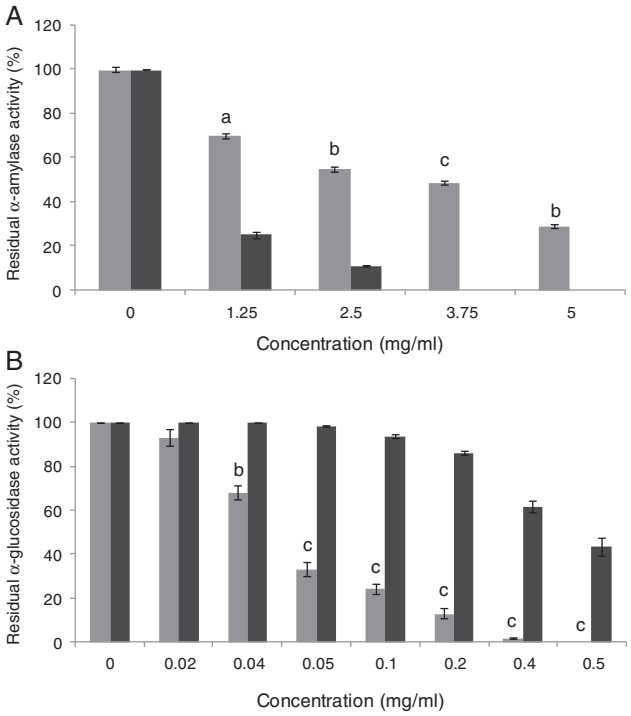

Fig. 2. Effect of *D. simplex* extract (▩) on α-amylase activity (A) and α-glucosidase activity (B). Data are presented as mean ± SD of triplicate determinations. Different letters above the bars indicate significant differences when compared with the standard antihyperglycemic agent acarbose (■): a ($p < 0.05$), b ($p < 0.01$) and c ($p < 0.001$).

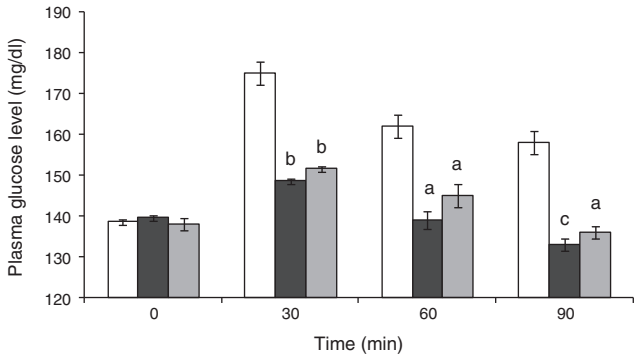

Fig. 3. Effect of the oral administration of *D. simplex* extract (▩) and the standard antihyperglycemic agent acarbose (■) on blood glucose level of maltose-loaded rats. Data are presented as mean ± SD of triplicate determinations. Different letters above the bars indicate significant differences when compared with to the control group (□): a ($p < 0.05$), b ($p < 0.01$) and c ($p < 0.001$).

or acarbose. Interestingly, the plant extract reached the highest α-glucosidase inhibitory activity at 0.4 mg/ml, and had even higher inhibitory activity than acarbose at this concentration. Based on IC_{50} values, the studied extract was more effective in inhibiting α-glucosidase (IC_{50} = 0.046 mg/ml) than α-amylase (IC_{50} 3.46 mg/ml), which could be of great pharmaceutical interest. In fact, it is possible that this difference in inhibition specificity would reduce some of the side effects such as diarrhea and flatulence associated with the classic drug acarbose used for DM treatment and which presented strong inhibition activity against α-amylase (Bischoff, 1994). The enzyme inhibitory activities of the extract of *D. simplex* flowers are probably due to its richness of phenolic compounds. In fact, results showed that ethyl acetate extract of *D. simplex* flowers present the highest phenolics and flavonoids contents as compared to leaves extract (data not shown). Falleh et al. (2013) also showed that *D. simplex* flowers exhibited the highest total phenolics and flavonoids contents followed by leaves. HPLC analysis showed that the main phenolic compound identified in *D. simplex* flowers was caffeic acid. Furthermore, epigallocatechin, chlorogenic, p-coumaric and 3,4-dimethoxybenzoic acids were also identified (Falleh et al., 2013). Manickam et al. (1997) reported that some phenolic compounds of the heartwood of *Pterocarpus marsupium*, significantly reduced blood glucose level of hyperglycemic rats. Moreover, Bansal et al. (2012) reported the antidiabetic effect of the flavonoid rich fraction of *Pilea microphylla* in a high-fat diet and streptozotocin-induced murine model of diabetes. In fact, they report that this flavonoid rich fraction is beneficial in controlling blood glucose level, abnormalities in lipid profiles and oxidative stress in diabetic mice.

In vivo *inhibitory effect on postprandial hyperglycemia*

During this experiment, ethyl acetate extract of *D. simplex* flowers was administered before an oral maltose or glucose tolerance tests to illustrate if this extract have an antihyperglycemic effect. Fig. 3 showed that after a maltose overdose, mice of the control group showed a blood glucose peak, which reached 175 mg/dl after 30 min time course of the experiment. The treatment with the standard antihyperglycemic agent acarbose significantly inhibited the rise in blood glucose levels of maltose-loaded mice, which reached 148 mg/dl after 30 min time course of the experiment. Interestingly, the group treated with *D. simplex* extract produced a hypoglycemic effect beginning at time-point 30 min comparable to acarbose. The blood glucose levels in acarbose-treated or *D. simplex* extract-treated mice remained significantly lower than those of control animals during 90 min time course of the experiment. Similarly, Andrade-Cetto and Wiedenfeld (2011) showed that *Opuntia streptacantha* extract produces an antihyperglycemic effect when administered to maltose-loaded rats, as compared to acarbose. These findings may result from α-glucosidase inhibition or insulin release stimulation, among other mechanisms involved in blood glucose-lowering effect. Therefore, a glucose tolerance test was also investigated and obtained results showed that treatment with *D. simplex* extract did not significantly inhibit the rise in blood glucose levels of glucose-loaded mice (data not shown). Consequently, these results suggest that ethyl extract of *D. simplex* flowers may produce postprandial antihyperglycemia through the inhibition of α-glucosidase activity in the intestinal tracts, which is in line with the *in vitro* study.

Conclusions

The trends toward natural ingredients and products promoting health are likely to increase. Data about *D. simplex* are very few. Therefore, improving knowledge on the composition, analysis and properties of *D. simplex*, would assist in efforts for functional applications of these plants as new potential health-promoting vegetable. *D. simplex* leaves and flowers contain several important nutrients such as macro-minerals, essential fatty acids and other valuable bioactive compounds known for their interesting biological properties. Interestingly, the ethyl acetate extract of *D. simplex* flowers is effective in inhibiting α-glucosidase activity and significantly reduce the rise in blood glucose levels of maltose-loaded mice as compared to the standard acarbose. These results suggest that *D. simplex* flowers could be used to delay the quick digestion of starch, which may reduce the peak blood glucose and therefore have potential as an antihyperglycemic agent. Further investigations would include the study of antidiabetic activity guided by the isolation of active compounds from *D. simplex* flowers.

Authors' contributions

HJ (PhD student) contributed in collecting the plant samples, to the chemical and biochemical analyses. BK and MC (PhD student) contributed to the Biochemical assays and to the postprandial hyperglycemia tests. SZ carried out analysis of lipid compounds. YBA contributed in critically revising the manuscript. NZ (thesis supervisor of HJ) participated in the study design and performed the redaction of the manuscript. All Authors have read and approved the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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