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Research Article

In vitro α-amylase Inhibitory Effect and Antioxidant Activity of *Teucrium barbeyanum* (A Libyan Ayurvedic Medicinal Plant)

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Abstract

Background: Diabetes mellitus is a metabolic disease characterized by high levels of blood glucose caused due to deficiency of insulin secretion or insulin action. The inhibition of carbohydrate hydrolysing enzymes such as α-amylase may be an important strategy in the decreasing of postprandial blood glucose in patients with type II diabetes (T2DM). *Teucrium barbeyanum* is an important traditionally used medicinal plant in Libya to manage T2DM. **Purpose:** The objective of the present study was to investigate the antioxidant activity and the *in vitro* pancreatic α-amylase inhibitory effect of *Teucrium barbeyanum*. **Methodology:** The methanolic extract of *Teucrium barbeyanum* was analysed for its content of polyphenols and flavonoids and the antioxidant capacity of the extract was determined by DPPH radical scavenging method using ascorbic acid as a positive control. Finally, the inhibition potency on α-amylase was assayed by CNPG3 method using acarbose as a positive control. **Results:** The extract has high antioxidant ($IC_{50} = 3.72 \pm 0.03$ µL mL^{-1}) and α-amylase inhibitory activities ($IC_{50} = 16.51 \pm 0.83$ µL mL^{-1}) when compared to ascorbic acid ($IC_{50} = 8.13 \pm 0.56$ µL mL^{-1}) and acarbose ($IC_{50} = 27.24 \pm 1.04$ µL mL^{-1}), respectively. **Conclusion:** These findings showed that the methanolic extract of *Teucrium barbeyanum* possesses α-amylase inhibitory activity and antioxidant potency which may provide a good source for natural products and may be used in the development of a new oral hypoglycemic agent.

Key words: *Teucrium barbeyanum*, *in vitro* α-amylase inhibitory activity, antioxidant capacity, Libyan medicinal plants, T2DM

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The hydrolysis of dietary starch is the main source of blood glucose and pancreatic α-amylase (E.C.3.2.1.1) is the major enzyme in the digestive system that is involved in the initial step that leads to the breakdown of starch. It is believed that the inhibition of pancreatic α-amylase can significantly lead to the retardation of glucose absorption and reduction in postprandial blood glucose level after a mixed diet of carbohydrate and therefore may be an important strategy in the management of hyperglycemia associated with T2DM and obesity[1]. Currently, there are many synthetic oral antidiabetic agents such as acarbose, miglitol and voglibose which act by competitively inhibition of α-amylase activity, while effective in reducing the high blood glucose levels in many patients. Unfortunately, continued use of these drugs is often associated with undesirable effects, such as liver toxicity and adverse gastrointestinal symptoms[2,3], as well as associated financial constraint. It is for these reasons that there is a need to search for potent and cheap alternatives, with little or no side effects.

Pancreatic cell damage caused by Reactive Oxygen Species (ROS) such as the superoxide anion radical (O^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) is a known etiology of T2DM[4-6]. A previous study reported that an elevated malondialdehyde content in pancreatic tissue of diabetic animal models caused by lipid peroxidation, the study also confirmed the role of free radicals in pathogenesis and progression of T2DM[7]. Recent interest in plant phytochemicals such as polyphenols, flavonoids and glycosides has focused on their potential benefits to human health, the phytochemicals have abilities not only to reduce oxidative stress but also to inhibit carbohydrate hydrolysing enzymes like α-amylase, thus preventing hyperglycemia[8-11]. Therefore, this phytochemicals generally possesses high antioxidants and antidiabetics potencies.

Teucrium barbeyanum, commonly called as 'Jaada' in Libya, is an endemic perennial herb that grows in Al-Jabal Al-Akhdar (The Green Mountain) in Libya[12]. It is rich in natural antioxidants mainly neo-clerodane diterpenoids[13-16]. A recent study also reported that *Teucrium barbeyanum* was rich in polyphenols mainly hydroxytetramethoxyflavones, salvigenin, chrysosplenetin, cirsilineol, cirsimaritin, cirsiliol, apigenin, luteolin, methyl caffeate and hydroxybenzaoic acid[17]. This plant is traditionally used in Libya as diuretic, diaphoretic, antiseptic, antipyretic, antispasmodic and hypoglycemic agents[17,18]. The hypoglycemic potential of *Teucrium* species has been evaluated in diabetic rats after its administration for two weeks[19]. Previous studies had shown the antidiabetic potential of *Teucrium* species but there is no information on the mechanism of action of this plant[17,20]. Therefore, the aim of the present study was to evaluate the biological activity of *Teucrium barbeyanum* as an antioxidant and α-amylase inhibition effect, as a possible mechanism of action of this plant in the management of hyperglycemia.

MATERIAL AND METHODS

Chemicals: Porcine pancreatic α-amylase (E.C.3.2.1.1), 2-Chloro-4-nitrophenol-α-D-maltotrioside (CNPG3), 2,2'-Diphenyl-1-picrylhydrazyl (DPPH), acarbose, dimethylsulfoxide, Foline Ciocalteu reagent, aluminum trichloride ($AlCl_3$), gallic acid and quercetin were purchased from Sigma-Aldrich Chemical Co., St Louis, MO, USA. All the solvents used were analytical grade.

Plant materials: The aerial parts of *Teucrium barbeyanum* were purchased from a local herbal and ayurvedic medicine store in Albayda city, Libya. The plant specimens were botanically authenticated by a competent botanist according to flora of Libya at the Department of Botany, Faculty of Science, Benghazi University and a voucher specimen of plant was kept in the department. The plant samples were washed by distilled water and air-dried in shade at room temperature (25°C) for three weeks to get a consistent weight and then stored in a plastic zip bag at 4°C until use.

Solvent extraction: The air-dried aerial parts of plant samples were finely powdered using liquid nitrogen. The hydro-alcoholic plant extracts were prepared by adding 10 g of dry powder in 100 mL of 85/15 (v/v) methanol/water at ambient temperature for 24 h with occasional stirring and shaking. The extract was filtered through a Whatmann filter paper No. 1. The extraction and filtration were remade for a second time. The second filtrate was mixed with the first. The clear filtrate was evaporated to dryness using a rotary evaporator (Heidolph WB 2000, UK) at low temperature (40°C) and pressure. The dried residue was dissolved in 10 mL of DMSO and kept at 4°C in air tight vials, till further use.

Determination of total phenol content: Total phenolic content of plant extract was determined using Folin-Ciocalteau assay by Slinkard and Singleton[21] with some modifications. Hundred microliters of appropriate dilutions of the extracts were oxidized with 1.0 mL 10% Folin-Ciocalteau's

reagent (v/v) the solution was mixed and after 5 min, neutralised by 1.0 mL of 2.0% sodium carbonate. The reaction mixture was incubated in the dark at room temperature for 60 min, absorbance of each mixture was recorded at 750 nm. Gallic acid (25-400 μg mL^{-1}) was used as reference standard. The results expressed as μg gallic acid equivalents per mg dry weight of extract (μg GAE mg^{-1}).

Determination of total flavonoid content: The total flavonoid content of the extracts was determined using a slightly modified method reported by Chang *et al.*[22] using a method based on the formation of a complex flavonoid-aluminium, having a maximum absorbance at 415 nm. Briefly, 250 μL of appropriately diluted sample was mixed with 750 μL ethanol, 50 μL 10% $AlCl_3$, 50 μL 1 M sodium acetate and 1.0 mL DMSO, followed by incubation in the dark at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm. Quercetin (25-200 μg mL^{-1}) was used as reference standard. The total flavonoid content was subsequently calculated as μg quercetin equivalents per mg dry weight of extract (μg QE mg^{-1}).

DPPH radical scavenging activity: The DPPH scavenging activity was measured according to the method described by Blois[23] with modifications. Briefly, in a 96 well microplate, 180 μL 6×10^{-5} M DPPH solution in methanol and 20 μL of plant extract at different concentrations were added. The reaction mixture was left in the dark for 30 min at 37°C and the absorbance was read at 517 nm against blank using multimode microplate reader (SpectraMax M2e, USA). Ascorbic acid was used as positive control. The inhibition of DPPH free radical scavenging activity in percent was subsequently calculated according to the equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Blank}}} \times 100$$

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration.

***In vitro* biochemical assay of α-amylase inhibitory activity:** The α-amylase inhibitory activity was estimated according to the slightly modified method of Gella *et al.*[24]. Briefly, the enzyme α-amylase solution was prepared by mixing of α-amylase in 100 mL of 40 mM sodium phosphate buffer, pH 6.9. Positive control, acarbose was obtained by dissolving in phosphate buffer. Twenty microlitter of enzyme, 80 μL of the plant extract in the concentration range of 2-200 μg mL^{-1} and 1 mL of CNPG3 were incubated for 5 min at 37°C. The absorbance was measured at 405 nm (Jenway 6405 UV/Visible, Great Britain). The control samples were also prepared accordingly without any plant extract/acarbose and were compared with the test samples containing various concentrations of plant extract. The results were expressed as inhibition percentage calculated by using the equation:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Concentration of extract resulting in 50% inhibition of enzyme activity (IC_{50}) was determined graphically.

Statistical analysis: All data points are Mean±Standard deviation of at least three independent experiments. The data were evaluated statistically using Student's t-test by Microsoft Excel 2007 (Microsoft Corporation, USA). The p-value of <0.05 was considered to indicate statistical significance.

RESULTS

The present study determines the antioxidant activity of the *Teucrium barbeyanum* methanolic extract and its *in vitro* inhibitory effect on α-amylase activity. The calculated %yield of extract from dry weight of areal part of the plant in 85% of methanol is shown in Table 1. The percent yield of crude extract following the removal of solvent using rotatory evaporator, was relatively high 23.75%. This indicated that substances in *Teucrium barbeyanum* were quite polar and lot of polar compounds dissolved in polar solvents.

Polyphenolic compounds such as flavonoids, phenolic acids and tannins are well known as effective free radical scavengers and antioxidant. Total polyphenolic compounds and total flavonoids in *Teucrium barbeyanum* methanolic extract were determined with a linear gallic acid standard curve ($y = 0.0035x-0.0148$, $r^2 = 0.9992$) for polyphenolic compounds and with a linear quarcetin standard curve ($y = 0.0054x+0.0209$, $r^2 = 0.9983$) for total flavonoids. The results indicated that total phenolic and total flavonoids contents were (52.78 μg GAE/mg dry extract weight) and (30.55 μg QE/mg dry extract weight), respectively (Table 1).

The assay of the scavenging of DPPH radical is widely used to evaluate the antioxidant capacity of extracts from different plant materials. The DPPH is one of the compounds

Table 1: Extraction yield, total polyphenols and total flavonoids contents of methanol extract from *Teucrium barbeyanum*

	Teucrium barbeyanum
Sample dry weight (g)	10
Extract dry weight (g)	2.375
Total yield (%W/W)*	23.75
Total polyphenols contents (μg GAE/mg dry extract weight)	52.78±1.65
Total flavonoids contents (μg QE/mg dry extract weight)	30.55±0.76

Values represent Means±Standard deviation of triplicate readings, *Total yield (%W/W) = (Extract dry weight/sample dry weight)×100, GAE: Gallic acid equivalents and QE: Quercetin equivalents

Table 2: IC_{50} values for *Teucrium barbeyanum* methanolic extract, ascorbic acid (positive control) and acarbose (positive control) in DPPH free radical scavenging and α-amylase inhibitory activity

Extract/positive control	DPPH IC_{50} (μL mL^{-1})	α-amylase inhibitory activity IC_{50} (μL mL^{-1})
Teucrium barbeyanum	3.72±0.03	16.51±0.83
Ascorbic acid	8.13±0.56*	-
Acarbose	-	27.24±1.04*

DPPH: 2,2'-Diphenyl-1-picrylhydrazyl, IC_{50} (μg mL^{-1}), concentration for scavenging or inhibiting activity 50% of DPPH radicals α-amylase, Values represent Means±Standard deviation of triplicate readings, *Values in same columns with star are statistically different ($p<0.05$), n = 3, Student's t-test was performed to analyze this data set

that possess a proton free radical and shows a maximum absorption at 517 nm. The percentage of inhibition of DPPH within the assay time will reflect the antioxidant capacity of the extract assessed. The IC_{50} of the positive control (ascorbic acid) was 8.13 μg mL^{-1}. *Teucrium barbeyanum* methanol exhibit a high percentage of inhibition with 91.98±0.009, which is higher than ascorbic acid (77.94±0.006). The IC_{50} value of the extract was 3.72 μg mL^{-1} as in Table 2. The study showed that the extract had a proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The extract was used within 0-80 μg mL^{-1} final concentrations in order to calculate the percent inhibition of α-amylase activity and IC_{50} value. The IC_{50} value was calculated by plotting the percentage inhibition of α-amylase activity versus concentration of *Teucrium barbeyanum* extract. It was found that the α-amylase activity decreased with increasing *Teucrium barbeyanum* extract concentration. Based on the results, 95.76% inhibition of α-amylase activity occur at higher dose of extract used with an IC_{50} value of 16.51 μg mL^{-1} (Table 2). Acarbose, the positive commercial control that is used for reducing glucose level in the blood. In this study, inhibit the α-amylase activity by 80.41% at the same higher dose of extract used with an IC_{50} value of 27.24 μg mL^{-1} (Table 2).

DISCUSSION

Clinically effective substances obtained from plants, including those not previously categorized as medicinal herbs. Recently, phytotherapy has often used to treat several diseases, besides modern medicine. A lot of natural extracts have proven beneficial as antidiabetic activities and are used to mange diabetes. Herbal extracts have been used perfectly or ultimately for the processing of numerous modern medicines[25-28].

Teucrium species is the most widespread plant in the world that is used for medical purposes. In traditional medicine, this plant is used as a diuretic and for the treatment of gout, rheumatism, hyperlipidemia, hyperglycemia, hypertension, spleen and liver disease[29-31].

The overall extraction yield of *Teucrium barbeyanum* was 23.75%. The study also revealed that the hydro-methanolic extract was rich in phenolic and flavonoids. This result is in agreement with previous study done by Alwahsh *et al.*[17]. Total polyphenols of the hydro-methanolic extract of *Teucrium barbeyanum* was estimated by the Foline Ciocalteu method as 52.78 μg GAE/mg dry extract weight. The total flavonoid content of the plant methanolic extract was estimated by using $AlCl_3$ colorimetric technique in terms of quercetin equivalents as 30.55 μg QE/mg dry extract weight. It has been acknowledged that phenolic and flavonoids content show significant antioxidant action on human health[32-34]. The polyphenolic compounds like flavonoids, phenolic acids and tannins are known to have an ideal chemical structure for effective free radical-scavengers activities that have shown to be more practical as antioxidants *in vitro* than vitamins E and C, this fact was in agree with the results[35,36]. Furthermore, a number of previous studies have demonstrated a strong correlation between phenolic content and antioxidant properties[37]. Scavenging antioxidants have a role in delaying or inhibiting the initiation or propagation of oxidative chain reaction, thus prevent or repair oxidative damage done to the body's cells by oxygen reactive species[38].

The free radical scavenging activity of *Teucrium barbeyanum* extract was determined by the DPPH test. This test aims to measuring the ability of the extract to scavenge the stable DPPH radical formed in solution by donation of a hydrogen atom or an electron[39]. The DPPH radical scavenging activities of *Teucrium barbeyanum* hydro-methanolic extract and ascorbic acid are shown in Table 2. Antioxidant activity plays a role in the treatment of diabetes mellitus, where it is

reported that the concentrations of antioxidants are reduced in diabetic patients[40-42]. Some *Teucrium* species have been reported to possess both antidiabetic activity as well as antioxidant activity[30]. That is why *Teucrium barbeyanum* is important in the Libyan folk medicine in the management of diabetes mellitus.

Although, there are documents of antihyperglycemic and antidiabetic activity of *Teucrium barbeyanum*[17,43], there are no previous reports on the activity of *Teucrium barbeyanum* on *in vitro* α-amylase activity.

In the present study, *Teucrium barbeyanum* was tested and found to possess favourable inhibitory effects on starch break down *in vitro*. The results as in Table 2 are in support of the ability of the *Teucrium barbeyanum* extract to inhibit amylase activity could be one of the mechanisms by which extract causes the reduction of blood glucose obtained in Libyan folk medicine. Natural compounds in plants especially polyphenols may attributed to have a potential antidiabetic effect because of their ability to inhibit the activities of digestive enzymes[44].

Various *in vitro* assays have shown that many plant polyphenols possess carbohydrate hydrolysing enzyme inhibitory activities[44,45]. Since these molecules exert antioxidant effect, it is likely that antioxidant and α-amylase inhibitory properties should come from polyphenolic contents. This can be an important dietary strategy to reducing postprandial hyperglycemia and the complications associated with use of synthetic α-amylase inhibitors in diabetes mellitus. For these, a drug development program should be undertaken to develop modern drugs with the compounds isolated from this plant.

CONCLUSION

Regarding the importance of α-amylase as a starch hydrolysing enzyme in the gastrointestinal tract, *Teucrium barbeyanum* extract was evaluated for its inhibitory effect on α-amylase activity along with its antioxidant efficiency. The results of present study indicated that *Teucrium barbeyanum* extract possess compounds that have antioxidant capacity and α-amylase inhibitory activity. These results also provide the motivation for further planning of clinical nutrition and diabetic research studies on bioactive compounds of plants and development of antidiabetic dietary products.

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