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In vitro antidiabetic assessment of *Ocimum forskolei* L growing in Saudi Arabia

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Abstract
The main objective of current study was to investigate the *in vitro* antidiabetic activity of *Ocimum forskolei*. *In vitro* antidiabetic assays such as inhibition of α-amylase enzyme, non-enzymatic glycosylation of hemoglobin were carried out. The results of α- amylase inhibition assay revealed that the inhibitory activity (IC_{50}) of both leaves and stems methanol extracts are almost the same (72.3 and 78.9µg/ml, for leaves and stems extracts, respectively) when compared with positive control (Acarbose IC_{50} value of 45.3 µg/m). Regarding the inhibition of glycosylation of hemoglobin, inhibitory activity (IC_{50}) both leaves and stems methanol extracts were (54.4 and 70.3µg/ml, for leaves and stems extracts, respectively) when compared the positive control, alpha-tocopherol (35.4µg/ml). The results from this study indicated that *Ocimum forskolei* leaves and stems methanol extracts showed considerable *in vitro* antidiabetic activity.

Keywords: *Ocimum forskolei* , antidiabetic, α-amylase, non-enzymatic glycosylation

Introduction
Diabetes is one of the most prevalent chronic diseases, that occurs when the pancreas cannot do its function efficiently to produce enough insulin or when the body cannot use the produced insulin properly. Eventually, this leads to an increased Blood glucose level (hyperglycemia). Diabetes is classified clinically as Type-1 characterized by insulin deficiency and Type-2 characterized by insulin inefficiency. Untreated Diabetes could lead to severe complications to the heart, blood vessels, eyes and kidneys [1, 2].
Medicinal plants are promising source of novel curing agents because of their potency and safety in comparison with those of synthetic origin. Saudi Arabia is one of the richest floras and contains thousands of medicinal plants. Most of these plants are reputed for their medical efficiency against many diseases [3-6].
The genus *Ocimum* (Family: Lamiaceae), is the well-studied genus of all the aromatic herbs and comprises around 160 species. *Ocimum*, is also famous by basil, grows to a height of about one meter and has opposite decussate arranged leaves and stems quadrangular in shape. This genus is commonly used in folk medicine as a tonic and alleviates mental fatigue, colds, spasms, rhinitis, wasp stings, and snakebites [7, 8].
Ocimum forskolei L is a perennial herb growing in Saudi Arabia. *O. forskolei* L is used in folkloric medicine as an herbal remedy for spasms and digestive agent as well as mosquito's repellent factor. Hence, there are some reports stated that some *Ocimum* species expressed anti-hyperglycemic and antioxidant activities [9-13]. These finding encouraged team to carry out the present study to investigate possible antidiabetic activity of *O. forskolei* which have not yet been reported.

Material and methods
Plant Material
The plant was purchased from local markets in Al-Ahsa region and will be air dried in shade will be kept for extracting the active ingredients. Voucher sample will be kept in the Department of Pharmacognosy, college of clinical pharmacy, King Faisal University (Apr-OF) [14].

Extraction and fractionation
The powdered air dried plant material (leaves and stems of *O. forskolei* 500.0 g and 300 g respectively) was exhaustively extracted twice at room temperature (each for 5 days) using 3l of 70% MeOH/H_2O applying cold maceration technique at room temperature to protect the potential active ingredients from being decreased or destroyed. The solvent mixture was removed through distillation under vacuum using Rota vapor and dried extracts

were directly freeze-dried to give dry extracts of leaves and stems, 40g and 16g respectively that were kept in -20°C for the next steps [15].

Chemicals

Acarbose, gentamycin, α-amylase from porcine pancreas and alpha-tocopherol were purchased from Sigma Aldrich (ST. Louis. Mo, USA). Solvents used for extraction and assays were all of analytical grade.

Preparation of O. forskolei stock solution

Various extracts of *O. forskolei* were dissolved in dimethylsulphoxide (DMSO), to prepare different concentration viz, 10, 20, 30 mg/ml in DMSO solution [16].

***In vitro* anti-diabetic models**

α-Amylase inhibitory activity

The assay mixture was prepared to contain 0.02M sodium phosphate buffer (200 µl), α-amylase enzyme (20 µl) together with different plant extracts in the range of concentrations 20-100 µg/ml. Then, it was incubated for 10 min at room temperature followed by the addition of 200 µl of 1% starch suspension to all the tubes containing reaction mixture. The reaction was later terminated by the addition of 400 µl of 3, 5 di-nitro salicylic acid (DNSA) color reagent. Then the tubes were kept in boiling water bath for 5 minutes, and later were kept till being cooled at room temperature and diluted with 15 ml of distilled water. The absorbance of each reaction mixture was measured at 540 nm. Control mixture reactions were also prepared accordingly without addition of extracts of plant under investigation and were compared with the test samples containing concentration of different plant extracts (20-100 µg/ml) freshly prepared in DMSO. The results were indicated as % of inhibition of activity using the following formula:

$$Inhibition\ activity\ (\%) = \frac{Abs(control) - Abs\ (extract)}{Abs\ (control)} X100$$

Where; Abs (control) is the absorbance of the control reaction (containing all reagents except the test sample) and Abs (sample) is the absorbance of different plant extracts [17]. The IC_{50} values (inhibitory concentration which will produce 50% inhibition of the enzyme activity) of the plant extracts were determined. Acarbose which is a well-known and safe anti-diabetic drug used to treat T2DM, was applied as a positive control in the concentrations ranged from 20 to100µg [18]. Experiments were achieved in triplicates

Non-enzymatic glycosylation of hemoglobin assay

Solutions of glucose (2%), hemoglobin (0.06%) and gentamycin (0.02%), were freshly prepared in phosphate buffer (0.01 M, pH 7.4). One ml of each of above mentioned solution was mixed. One ml of each concentration of different plant extracts (20-100µg/ml) was added to the prepared mixture. Then, the test tubes containing reaction mixture were incubated in dark place at room temperature for three days. After, the degree of glycosylation of hemoglobin was obtained colorimetrically at 520 nm where the percentage of inhibition was calculated applying this formula:

$$Percentage\ of\ inhibition\ = \frac{Abs(control) - Abs\ (extract)}{Abs\ (control)} X100$$

Where; Abs (control) is the absorbance of the control reaction (containing all reagents except the test sample) and Abs (sample) is the absorbance of different plant extracts. The IC_{50} values (inhibitory concentration which will produce 50% inhibition of the enzyme activity) of the plant extracts were determined. Alpha-Tocopherol was used as a standard drug [17, 18]. Experiments were carried out in triplicates

Results and discussion

A-Amylase inhibitory activity

The *in vitro* α- amylase inhibitory measurements demonstrated that both leaves and stems methanolic extracts of *O. forskolei* have potential of α- amylase inhibitory activity. α-Amylase inhibitory activity was compared based on the calculated IC_{50} values (Table 1). However, the observed α-amylase inhibitory activity of both leaves and stems extracts are almost the same (72.3 and 78.9µg/ml, for leaves and corollas extracts, respectively). Acarbose used as the positive standard showed IC_{50} value of 45.3µg/ml under similar conditions. Both leaves and stems methanol extracts of *O. forskolei* showed promising result in α-amylase inhibition assay, suggesting that *O. forskolei* might be effective in slowing down hydrolysis of polysaccharides like starch to glucose.

Table 1: α- Amylase inhibitory effect of *O. forskolei.*

	percentage of inhibition		
conc. µg/ml	**leaves extract**	**stem extract**	**standard (Acarbose)**
20	16.4±1.8	10.2±1.8	32.2±***1.8***
40	25.3±1.9	19.0±1.5	43.8±***2.3***
60	47.5±3.1	44.7±1.7	64.9±***4.0***
80	55.8±3.6	50.8±1.4	75.5±***2.4***
100	65.5±1.8	56.4±2.5	81.1±***2.3***
IC_{50} µg/ml	72.3	78.9	***45.3***

Values were expressed as mean ± SD (Standard Deviation) n=3 independent experiments

Non-enzymatic glycosylation of hemoglobin assay

The inhibitory activities of both leaves and stems extracts of *O. forskolei* were compared according to their IC_{50} values (Table 2) **.**Leaves methanol extract showed a comparable value of IC_{50} (54.4µg/ml) to the positive control, alpha-tocopherol (35.4µg/ml). Stems methanol extract showed relatively distinct IC_{50} (70.3µg/ml) when compared to that of standard under the same conditions. The current study revealed a good activity of leaves methanol extract in preventing such binding of glucose to surface proteins of erythrocytes. On the other hand, stems methanol extract expressed low activity when compared to positive control.

Table 2: Non-enzymatic glycosylation of hemoglobin effect by *O. forskolei.*

	Percentage of inhibition		
conc. µg/ml	**leaves methanol extract**	**stem methanol extract**	**standard (alpha-Tocopherol)**
20	34.5±4.0	23.2±2.3	38.8±***0.9***
40	39.8±2.8	37.1±1.4	49.3±***1.0***
60	50.6±1.0	46.2±1.5	71.6±***1.1***
80	66.8±2.0	53.1±2.3	81.0±***1.7***
100	72.9±2.6	64.8±2.8	82.7±***2.7***
IC_{50} µg/ml	54.4	70.3	35.4

Values were expressed as mean ± SD (Standard Deviation, n=3 independent experiments

Conclusion

The conducted *in vitro* examinations revealed a substantial α-amylase inhibitory and inhibition of glycosylation of hemoglobin for both leaves and stems methanol extracts of *O.*

forskolei. The present data illustrates that the methanol extracts for both leaves and stems of *O. forskolei* have good properties in treating diabetes. A confirmatory *in vivo* study is recommended to evaluate the potential hypoglycemic effect.

Conflict of interest
The authors declare no conflict of interest is associated with this work.

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