

Metabolite profiling of *Cycas revoluta* leaf extract and docking studies on alpha-glucosidase inhibitory molecular targets by phytochemicals

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Abstract: The leaves of *Cycas revoluta* were explored for their antioxidant, α -glucosidase and α -amylase inhibitory properties to develop safe and diet based therapeutic treatment of diabetes. In optimized fractionation, 60% ethanol provided the highest extract yield of $19.35 \pm 0.05^a\%$, TPC 95.70 ± 1.60^a mg GAE/g and TFC 55.60 ± 1.20^a mg Rutin/g extract. The antioxidant and anti α -glucosidase activities of 60% ethanolic extracts were also promising and statistically significant as compared with remaining plant extracts. Ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-Q-TOF-MS/MS) analysis of the leaf extract revealed the presence of three apigenin derivatives, kaempferol derivative, hexadecenoic acid and citric acid. The binding energy values of molecular docking studies supported the synergistic behavior of leaf extract to inhibit α -glucosidase activity. The leaves of *Cycas revoluta* were proved to be apigenin rich natural pool of metabolites of antidiabetic importance to improvise food functionalities.

Keywords: Antidiabetic, *Cycas revoluta*, optimized fractionation, phytochemical profiling.

INTRODUCTION

The functional foods are gaining importance due to their medicinal attributes usually governed by natural bioactive substances. The functional foods are emerging as an excellent and novel tool to prevent or treat chronic ailments including diabetes (Alkhatib *et al.*, 2017). Diabetes mellitus is a metabolic dysfunction results in consistent hyperglycemic conditions. Diabetes mellitus type II (DM-T2) is the most common type of diabetes growing at faster rate over the globe (Kirkman *et al.*, 2012). The number of diabetic patients in the world is expected to become double in the next decade (Olokoba *et al.*, 2012).

The DM-T2 pathogenesis is a chronic ailment and is also associated with detrimental health hazards and side complications including renal disorders, retinal damages and cardiovascular damages (Chaudhury *et al.*, 2017). The role of oxidative stress due to robotic life style, dietary habits and lack of exercise cannot be ignored in diabetes pathogenesis as scientific evidences are available in this context (Boden *et al.*, 2015; Asmat *et al.*, 2016; Pham-Huy *et al.*, 2008). Elimination of oxidative stress is imprinted in the form of physiological betterment and reduction in intensity of DM-T2 (Karam *et al.*, 2017). Many synthetic drugs are available for diabetes treatment but their side complications emphasize on the need of safe therapeutic agents (Kooti *et al.*, 2016). Plants may serve the purpose, being rich source of natural antioxidants and

α -glucosidase inhibitors. The anti α -glucosidase agents slow down the activity of this enzyme to delay the degradation of carbohydrates restricting the postprandial blood glucose level to rise (Brunetti and Kalabalik, 2012). Besides this, plants are advantageous as they are safe, cheap and easily available. Antioxidant rich diets and fortified foods with natural plant extracts may provide a successful food-based antidiabetic protocol which would not only prevent the pathogenesis but also stop disease prolongation.

Cycas revoluta (*C. revoluta*) is the wide spread genus of family *Cycadaceae* (Moawad *et al.*, 2010). The male and female cones of *C. revoluta* are known to have aphrodisiac, narcotic and stimulant effects (Kumar *et al.*, 2013). The use of this plant in folk medicines to treat diabetes provoked to explore it for antidiabetic potential to obtain nutraceutical leads for functional food development.

MATERIALS AND METHODS

Extract preparation

Liquid nitrogen treated fresh leaves were extracted with 20%, 40%, 60%, 80% and 100% ethanol using ultrasonication (soniprep 150). The excess solvents were removed on rotary evaporator and extracts were further lyophilized on freeze-dryer (Christ Alpha 1-4 LD (Germany)). The extract yields (%) were calculated and dry extracts were stored at -80°C till further use.

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Determination of total phenolic and flavonoid contents

The total phenolic contents were determined by Folin Ciocalteu reagent-based method and results were expressed as results were expressed as gallic acid equivalent (GAE) mg/g plant extract (Zengin *et al.*, 2010). Total flavonoid contents were determined by AlCl₃ based method. The absorbance of sample was taken at 510 nm and results were expressed as rutin equivalent mg/ g plant extract (Zhishen *et al.*, 1999).

Free radical scavenging activity

Briefly, 25% methanolic DPPH and plant extracts in concentration (50-200µg) were dissolved in methanol and stayed for 35 minutes. The absorbance was noted at 517 nm and free radical scavenging potential was calculated using the following formula (Chew *et al.*, 2011).

$$\% \text{ DPPH inhibition} = \frac{\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{blank}}} \times 100$$

Inhibition of alpha-glucosidase

In vitro antidiabetic potential of extracts was determined according to established method with little modification. The absorbance was measured at 405 nm. All the measurements were made in triplicate (Jabeen *et al.*, 2013). Following formula was used to calculate percentage inhibition,

$$\% \text{ inhibition} = \frac{A_b - A_s}{A_b} \times 100$$

A_b is absorbance of blank and A_s is absorbance of sample. Acarbose was used as standard reference and results were represented as IC₅₀ (µg/mL).

UHPLC-Q-TOF-MS/MS analysis

Chemical profiling in 60% ethanolic extract was carried out by UHPLC-Q-TOF-MS-MS on AB Sciex 5600-1 equipped with Eksigent UHPLC system. ThermoHypersil Gold Column (100mm × 2.1mm × 3µm) and gradient mobile phase (water and acetonitrile, each having 0.1% formic acid and 5 mM ammonium formate) was used at flow rate of 0.25mL/min, Ion spray voltage -4500 V, desolvation temperature 500°C).

Docking studies

Docking studies were carried out by using Molecular Operating Environment (MOE 2016.08). For α-glucosidase, docking studies were carried out on homology modelled α-glucosidase reported by our research group (Ali *et al.*, 2018). The 3D protonation, energy minimization and determination of binding site were carried out by our previously reported methods (Ifthikhar *et al.*, 2018; Ali *et al.*, 2018).

RESULTS

The 60% ethanol yielded maximum amount of extract, TPC and TFC followed by 80% ethanol. Statistical comparison indicated that extract yield (19.27 ± 0.07 %), TPC (125.33±1.28^a mg GAE/g PE) and TFC (58.20± 1.34

a mg RE/ g PE) were significantly higher among all extracts (p<0.05). The findings of DPPH assay and α-glucosidase inhibition showed that minimum IC-50 values for both activities were computed for 60% ethanol extract and significantly higher than remaining extracts (p<0.05). However, no extract could match the antiradical activity of BHA and enzymatic inhibition by acarbose (p<0.05). The all finding are summarized as table 1.

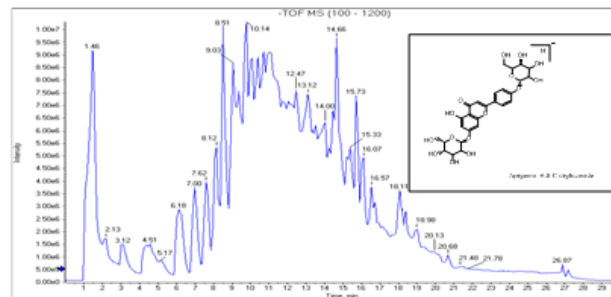


Fig. 1: Chromatogram of UHPLC showing peaks at various retention times.

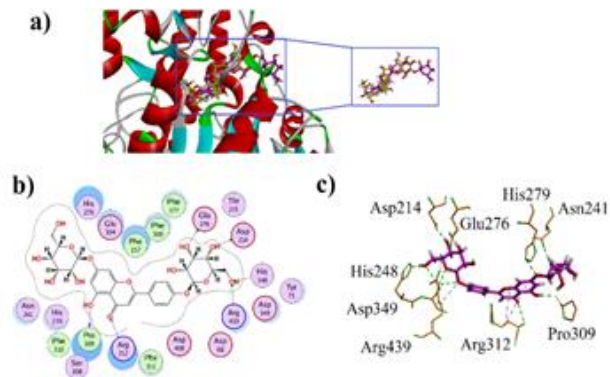


Fig. 2: (a) Three-dimensional diagram of apigenin -6,8-C diglucoside superposed over acarbose into the active site of homology modeled α-glucosidase.; (b) Two-dimensional interaction plot generated *via* MOE software showing interactions with the active site residues. c) Close-up depiction of the lowest-energy three-dimensional (3-D) docking pose of the compound.

The main spectrum of UHPLC-QTOF-MS-MS analysis is given as fig. 1. The detail of identified compounds along with analytical data of UHPLC-QTOF-MS-MS is given in table 2. The citric acid, apigenin and kaempferol derivatives were the potential metabolites identified in extract.

The results of molecular docking studies are represented as fig. 1. For docking studies, we used our previously reported homology modelled yeast α-glucosidase. Three-dimensional diagram of apigenin -6,8-C diglucoside superposed over acarbose is shown in fig. 2a. Two dimensional and 3D interaction plot of the compound is shown in fig. 2 b-c. The fig. 2 b-c revealed that the compound has shown six hydrogen bonding interactions

Table 1: Extract yields (%), TPC, TFC, DPPH assay and α -glucosidase inhibition by leaf extracts of *Cycas revolute*

Solvent composition	Extract yield (%)	TPC in mg GAE/g PE	TFC in mg RE/g PE	DPPH assay (IC-50 μ g/mL)	α -glucosidase inhibition (IC-50 μ g/mL)
100 % wáter	09.72 \pm 0.02 ^d	28.50 \pm 0.33 ^f	5.12 \pm 0.01 ^e	155.88 \pm 3.843 ^f	138.50 \pm 2.54 ^g
20% Ethanol	11.05 \pm 0.04 ^{cd}	50.25 \pm 1.07 ^e	15.22 \pm 0.14 ^d	141.56 \pm 2.98 ^e	108.12 \pm 3.57 ^f
40% Ethanol	14.25 \pm 0.05 ^c	70.85 \pm 1.05 ^c	38.77 \pm 0.56 ^b	120.93 \pm 3.12 ^d	88.34 \pm 2.50 ^e
60% Ethanol	19.27 \pm 0.07 ^a	125.33 \pm 1.28 ^a	58.20 \pm 1.34 ^a	78.10 \pm 2.34 ^c	66.80 \pm 2.45 ^b
80% Ethanol	17.11 \pm 0.05 ^{ab}	83.74 \pm 1.62 ^b	40.42 \pm 0.96 ^b	93.85 \pm 1.77 ^b	80.05 \pm 2.03 ^c
100% Ethanol	13.68 \pm 0.05 ^c	66.77 \pm 1.60 ^{cd}	27.30 \pm 0.65 ^c	94.33 \pm 1.82 ^c	85.77 \pm 1.90 ^d
BHA	-	-	-	35.35 \pm 0.33 ^a	-
Acarbose	-	-	-	-	30.25 \pm 0.09 ^a

Table 2: Mass spectrometric data for identification of metabolites in leaf extract under negative mode.

S. No	Name of Compound	Rt (min)	Molecular ion peak (m/z)	Main fragments ion (m/z)	Molecular formula
1	Citric Acid	1.411	191	191, 111, 87	C ₆ H ₈ O ₇
2	Apigenin C-hexoside C-hexoside isomer	7.940	593	575, 503, 473, 365, 383, 353, 325	C ₂₇ H ₃₀ O ₁₅
3	Unknown	8.545	439	393, 247, 265, 205, 163, 89	C ₁₈ H ₃₂ O ₁₂
4	Apigenin C-hexoside C-hexoside	8.595	593	503, 473, 383, 353	C ₂₀ H ₃₄ O ₂₀
5	Apigenin-6,8-C-diglucoside	9.198	593	473, 413, 311, 293	C ₂₀ H ₃₄ O ₂₀
6	Octadecenedioic acid	14.465	312	311, 293, 213, 183, 58	C ₁₈ H ₃₂ O ₄
7	Kaempferol derivative	19.700	654	653, 487, 447, 285, 79	C ₂₉ H ₅₀ O ₁₆

with the active site amino acid residues. It interacts with Asp214, Glu276, Pro309, Arg312, His 348 and Arg439. The computed binding affinity of the compound is -8.4839 kcal/mol. While the computed binding of standard drug acarbose is -9.2756kcal/mol.

DISCUSSION

The solvent polarity and ultrasonication along with freeze drying played an important role to enhance the extract yield which resultantly improved the TPC and TFC. These phenolics were reported to be the functional ingredients of plants (Mahmoudi *et al.*, 2016). Some previously published peer reviewed reports complemented the efficacy of 60% ethanol for enhanced extraction and improved biological activities (Al-Zuaidy *et al.*, 2016; Raza *et al.*, 2018). Natural α -glucosidase inhibitors in plant extracts are responsible for postprandial glucose level attenuation and these are usually secondary metabolites. The UHPLC-MS-MS analysis presented the 60% ethanolic extract as rich source of apigenin derivatives. The citric acid, Apigenin C-hexoside C-hexoside (isomers), Apigenin-6,8-C-diglucoside, Octadecenedioic acid and kaempferol derivative were the

identified compounds. Apigenin is well known for its α -glucosidase inhibitory properties. A kinetic study revealed that apigenin reversibly bound with α -glucosidase near to its active site to reduce its activity through hydrophobic interactions (Zeng *et al.*, 2016). The findings of current molecular docking revealed the hydrogen bond based functional interaction of apigenin -6, 8-C diglucoside at six active sites of α -glucosidase. These interactions and binding energy values confirmed the α -glucosidase inhibitory properties of *C. revolute* leaf extract. Hence diets rich in apigenin and kaempferol derivatives (Zang *et al.*, 2011) might be an appropriate choice to minimize disease pathogenesis and prolongation.

CONCLUSION

C. revolute leaves were proved as rich source of natural metabolites of nutra-pharmaceutical nature and might be exploited for the development of functional foods with antidiabetic features. The dietary value addition with apigenin and kaempferol regimes might be an appropriate strategy to develop antidiabetic functional foods. This will not only enhance the medicinal food quality but also reduce the socioeconomic burden developed by DM-T2

by utilizing the findings. However, careful *in vivo* trials may be conducted to further proceed for the development of antidiabetic agents.

ACKNOWLEDGMENTS

Dr. Umer Rashid has purchased MOE 2016.08 license under HEC-NRPU, Pakistan project 5291/Federal/NRPU/R&D/HEC/2016.

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