Antioxidant activity, Hypoglycemic potential and metabolite profiling of *Hyophorbe indica* leaf extract

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Abstract: Current work was performed to evaluate antioxidant activity, α-glucosidase inhibition, phytochemical profiling and *in vivo* hypoglycemic activity of freeze dried, ultrasonicated *Hyophorbe indica* leaf extracts. The highest total phenolic contents of 208.77±2.11 mg GAE/g DE and total flavonoid contents of 173.90±2.30 mg Rutin/g DE were obtained in 60% ethanol extract. The 60% ethanol extract exhibited maximum DPPH radical scavenging with IC_{50} value of 35.35±0.189µg/mL and total antioxidant power of 330.26±3.13 (ASE/g PE), respectively. The highest α-glucosidase inhibition (IC_{50} 36.52 ± 0.08µg/mL) was also observed for 60% ethanol extract. The 60% ethanol extract at dose of 450 mg/kg body weight reduced blood glucose level of alloxan induced diabetic mice by 51.41% which was quite comparable with metformin (56.67%). Twelve compounds namely citric acid, procyanidin B3, epicatechin, procyanidin B2, catechin, catechin derivative, procyanidin B1, apigenin-c-hexocide-c-hexocide, kaempferol, kaempferol derivative, quinic acid derivative and gallic acid have been identified by using UHPLC-Q-TOF-MS/MS in 60% ethanol extract.

Keywords: Antioxidant, anti-α-glucosidase, UHPLC-Q-TOF-MS/MS, *Hyophrobe indica*.

INTRODUCTION

Diabetes mellitus type 2 (DM2T) is the metabolic condition which is characterized by consistently elevated blood glucose due to impaired pancreatic function and insulin resistance. It is estimated that about 90% diabetic patients are suffering from DM2T and current expansion rate of this disease may result in 592 million patients by 2035 (Raz*et al.*, 2013; Guariguata *et al.*, 2014; Schwartz *et al.*, 2016). The current scenario of DM2T expansion reflected this disease as socio-economic burden due to heavy expenditures on its management and cure in many countries (Pari and Saravanan, 2007). The sedentary life style and eating habits, high caloric foods and stress are the major contributors in the DM2T expansion. These factors produce reactive oxygen species (ROS), excess of which may lead to oxidative stress. The role of ROS and oxidative stress in DM2T initiation and prolongation is evident from previously reported scientific studies (Pizzino *et al.*, 2017; Raza *et al.*, 2018). Synthetic drugs to manage diabetes are very effective but their side effects are very hazardous to human health. The serious drawbacks of synthetic drugs develop the need to identify alternate therapeutic modes for DM2T management. Plants are rich sources of safe, cheap and effective phytochemicals of medicinal importance to treat common chronic ailments. These natural bioactive compounds of plants are also competent α-glucosidase inhibitors. The α-glucosidase inhibitors resist the carbohydrate digestion in intestine and linger down their absorption, hence reduces the blood glucose concentration (Rouzbehan *et al.*, 2017). The antioxidant potential of phytochemicals in plants also combats elevated ROS levels in body to reduce the risks of DM2T progression (Zaid *et al.*, 2015). The identification of plants rich in potent α-glucosidase inhibitors is a reliable and cost effective approach to control hyperglycemia in natural way.

Family *Arecaceae* is comprised of more than 189 genera, 3000 species and some of them are well known for the presence of strong antioxidants and other biologically active agents. Plants from family *Arecaceae* remained under study for their pytotherapeutic effectiveness but still many species are away from the canvas in this regard. *Hyophorbe indica* (*H. indica*) belongs to family *Arecaceae* (Govaerts and Dransfield, 2005; Elgindi *et al.*, 2016). This plant is used to treat chronic ailments including diabetes by traditional practitioners. In spite of effective role of *H. indica* in folk medicine system to treat diabetes, yet no scientific evidence was reported on its biological activities and phytochemical screening. The current work was performed to confirm the antioxidant activity and antidiabetic potential of *H. indica*. The secondary metabolites in leaf extract, usually responsible for medicinal attributes were also profiled by UHPLC-Q-TOF-MS/MS.

MATERIALS AND METHODS

Green extract preparation

Fresh leaves of *H. indica* were quenched in liquid nitrogen and grinded to fine powder which was

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lyophilized using a freeze-dryer (Christ Alpha 1-4 LD (Germany). The freeze dried powder was dipped in hydroethanol solvent fractions (Ethanol, 100%, 80%, 60%, 40%, 20%) for 48 hours. Mixtures were further sonicated at soniprep 150. Obtained samples were shaken for 2 hours and filtered. The excess solvent from filtrate was removed under vacuum on rotary evaporator at 40°C. The extracts were again freeze dried for 48 hours. Extract yields (%) were calculated and extracts were stored at -80 °C till further use.

Determination of total phenolic and flavonoid contents

Total phenolic contents (TPC) of freeze dried leaf extracts were determined by Folin Ciocalteu reagent method. Results were expressed as gallic acid equivalent (GAE) mg/g dried extract (Zengin *et al.,* 2010). Total flavonoid contents (TFC) were determined by $AlCl_3$ colorimetric method. The results were expressed as rutin equivalent mg/g dried extract (Zhishen *et al.,* 1999).

Antioxidant activities

Antioxidant potential of extracts was determined by DPPH scavenging assay as reported previously with little modification (Fki *et al.,* 2005). Phosphomolybdenum complex formation method was used as per previously reported method with minute modifications to determine total antioxidant power (Prieto *et al.,* 1999). Ascorbic acid standard curve was drawn and results were represented as ascorbic acid equivalent per gram dried plant extract (ASE/g DE).

Anti-α-glucosidase activity

Inhibition potential of extracts against α-glucosidase was measured to evaluate *in vitro* antidiabetic potential. Acarbose was used as standard reference and results were represented as IC_{50} (µg/mL) values for each extract (Jabeen *et al.,* 2013).

UHPLC-Q-TOF-MS/MS analysis

The filtered extract sample was subjected to UHPLC-Q-TOF-MS/MS (AB Sciex 5600-1, equipped with Eksigent UHPLC system). The scanning range of 50-1200 m/z for MS/MS (negative ionization mode), column Thermo Hypersil Gold (100 mm× 2.1 mm × 3 µm), gradient mobile phase composition (water and acetonitrile) each having 0.1% formic acid and 5 mM ammonium formate, gradient programming started from 10% acetonitrile to 90% acetonitrile with mobile phase flow rate of 0.25mL/min was used.

In-vivo hypoglycemic activity

The mouse model was adopted to evaluate the *in vivo* antidiabetic effect of plant extracts. Briefly 30 healthy mice (8 week age) were selected and placed in animal house for ten days at 28°C±2.0°C temperature and relative humidity of 68.75% ±4.5% for adaptation to new environment. The prior permission from ethical committee of GC University Lahore was obtained to carry the trials. After acclimatization, mice were weighed and injected with alloxan at 150 mg/ kg body weight in 24 hours fasted mice. The blood glucose level (mg/dL) was measured by glucometer to check the diabetic status of mice. Mice having blood glucose level >200 mg/dL were considered as diabetic (Emordi *et al.,* 2016). The mice were divided into 5 groups each comprising of 6 mice. These groups were labelled as NG (normal group), DG (diabetic group), MG (metformin treated group, 250 mg/kg body weight), HG (half extract dose group, 250 mg/kg body weight) and FG (full extract dose group, 450 mg/kg body weight). Blood glucose levels were measured on weekly basis for 28 days.

STATISTICAL ANALYSIS

The experimental findings were evaluated for statistical significance by using Statistix 10.0 software. Analysis of variance (ANOVA) was used to compare variations in treatment to assess efficacy of trials.

RESULTS

Extract yields (%), TPC and TFC

The findings regarding extract yields, TPC and TFC are presented in table 1. Maximum extract yield 22.63 ± 0.19^{a} % was obtained when extracted with 60% ethanol followed by 19.07 ± 0.16^{b}%, extracted with 40%. Maximum TPC of 208.77 ± 2.11^{a} mg GAE/g DE and TFC 173.90 ± 2.30^{a} mg Rutin/g DE were obtained with 60% ethanol. Efficiency of 60% ethanol was significantly higher than other solvent fraction for high extract yield, TPC and TFC respectively ($\rho < 0.05$). Results have been represented with standard deviation values (±) along with significant level indicated by letter superscript.

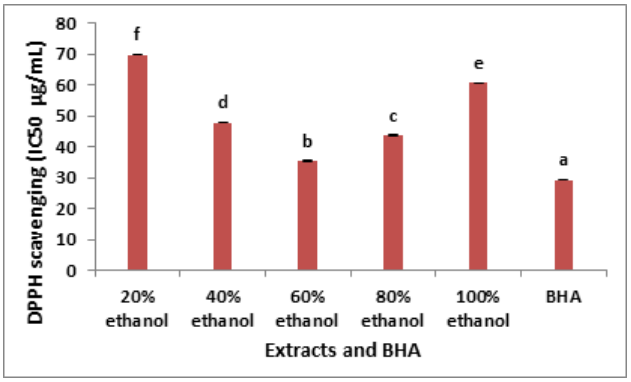

Fig. 1: DPPH scavenging activity of plant extracts and BHA.

Antioxidant activities

The IC_{50} values of extracts regarding DPPH radical scavenging in comparison with standard antioxidant BHA are given in fig. 1. Among extracts, maximum radical scavenging was exhibited by 60% ethanol extract with

IC_{50} value of 35.35±0.189µg/mL. This value is slightly less than BHA. The values having different letters were considered as significantly different (ρ<0.05). The results of total antioxidant power assay are represented in fig. 2. Findings unveiled that 60% ethanol fraction possessed maximum antioxidant power with value of 330.26±3.13[a] (ASE/g PE). Statistical analysis revealed that 60% ethanol extract exhibited significantly higher potential than remaining extracts (ρ<0.05).

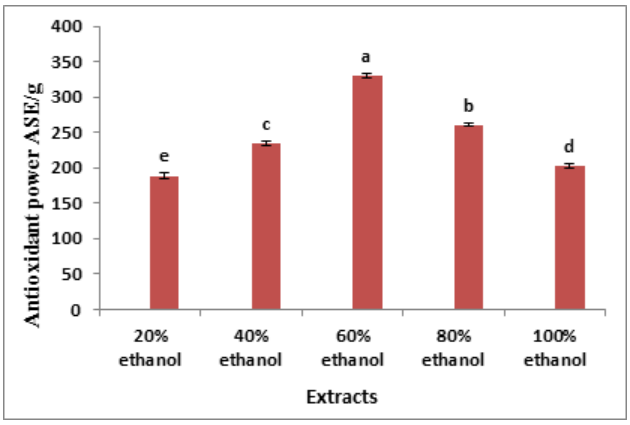

Fig. 2: Antioxidant power (ASE/g PE) of plant extracts

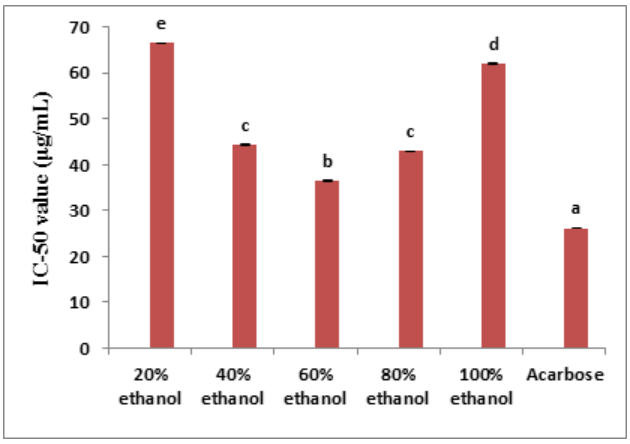

Fig. 3: Anti-α-glucosidase potential of extract fractions and acarbose.

Anti-α- glucosidase activity

In vitro antidiabetic potential of extracts was determined by measuring the extent of inhibition of α-glucosidase enzyme. The IC_{50} values (µg/mL) of plant extracts and standard enzyme inhibitor, the acarbose are presented in fig. 3. The comparison of extracts indicating that 60% ethanol extract exhibit least IC_{50} value for α-glucosidase inhibition which is 36.52±0.08µg/mL. However, acarbose exhibited lowest IC_{50} value, significantly higher than extracts (ρ<0.05).

UHPLC-Q-TOF-MS/MS analysis

Due to high efficiency of 60% ethanol extract regarding antioxidant and α-glucosidase inhibition, this fraction was subjected to UHPLC-Q-TOF-MS/MS analysis for metabolite identification. The identified compounds along with their retention time (Rt), fragment ions and molecular formula are listed in table 2.

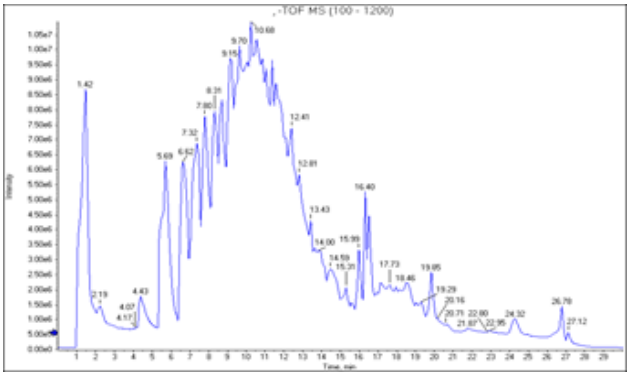

Fig. 4: Chromatogram of *H. indica* (UHPLC).

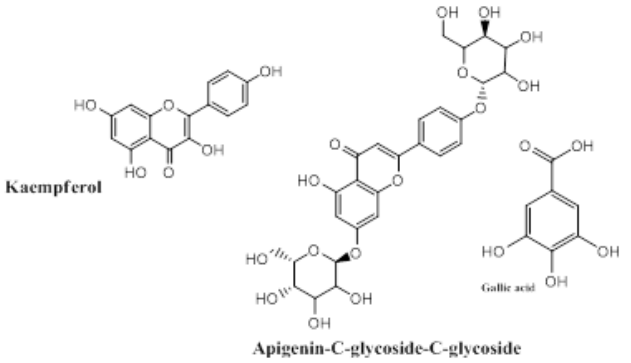

Fig. 5: Structures of Apigenin C-glucoside-C-glucoside, kaempferol and gallic acid.

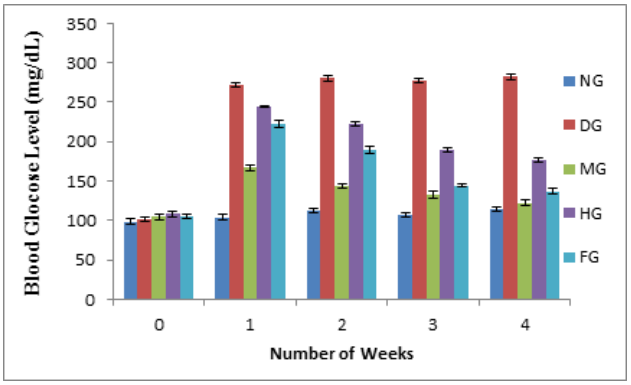

Fig. 6: Hypoglycemic potential of 60% ethanol extract and metformin in diabetic mice.

The chromatogram of UHPLC separation is shown in fig. 4 which indicated the peaks corresponding to metabolites elute from column at different retention times. These peaks were subjected to mass spectrometer for fragmentation to elucidate the structures of compounds. The structures of few identified compounds are given in fig. 5.

In-vivo hypoglycemic activity

The results of *in vivo* hypoglycemic activity are given in fig 6. The highest reduction in elevated glucose level was

Table 1: Extract yields, TPC and TFC from leaves of *H. indica*

Solvent composition	Extract yield (%)	TPC in mg GAE/g DE	TFC in mg Rutin/g DE
20% Ethanol	15.53 ± 0.07 [d]	105.55 ± 1.05 [e]	95.75 ± 1.25 [d]
40% Ethanol	19.07 ± 0.16 [b]	154.50 ± 2.15 [b]	118.11 ± 1.01 [b]
60% Ethanol	22.63 ± 0.19 [a]	208.77 ± 2.11 [a]	173.90 ± 2.30 [a]
80% Ethanol	18.46 ± 0.07 [c]	140.44 ± 2.09 [c]	117.60± 1.78 [b]
100% Ethanol	15.55 ± 0.06 [d]	119.24 ± 1.54 [d]	102.88 ± 1.22 [c]

Table 2: Peak assignments for identified compounds by UHPLC-MS/MS in negative mode

Sr. No	Name of Compound	Rt (min)	Molecular ion peak (m/z)	Main fragments ion (m/z)	Molecular formula
1	Citric acid	1.482	191	173, 129, 111	$C_6H_8O_7$
2	Procyanidin B3	5.438	577	451, 425, 407	$C_{23}H_{30}O_{17}$
3	Epicatechin	5.749	289	245, 263, 123	$C_{15}H_{14}O_6$
4	Procyanidin B2	6.557	577	451, 425, 407	$C_{23}H_{30}O_{17}$
5	Catechin derivative	6.695	933	577, 425, 355, 337, 209, 191, 147, 85	$C_{38}H_{46}O_{27}$
6	Procyanidin B1	7.222	577	451, 425, 407	$C_{23}H_{30}O_{17}$
7	Catechin	7.511	289	271, 245, 203, 123	$C_{15}H_{14}O_6$
8	Apigenin-c-hexocide-c-hexocide	8.380	593	503, 473, 395, 383, 353, 325	$C_{20}H_{34}O_{20}$
9	Kaempferol	9.114	285	217, 175, 151, 133	$C_{15}H_{10}O_6$
10	Kaempferol derivative	9.585	527	447, 364, 285, 241	$C_{17}H_{20}O_{19}$
11	Quinic acid derivative	11.341	733	653, 353, 299, 285, 191	$C_{32}H_{30}O_{20}$
12	Gallic acid	21.401	169	125, 97, 79	$C_7H_6O_5$

observed in MG (metformin treated group), while HG also exhibited substantial reduction in blood glucose level but slightly less than MG.

DISCUSSION

The polarity of the solvent system set by its chemical components for extraction was proved as the decisive factor for enhanced extract production. Interaction of solvent molecules with structural features of bioactives played important role in extraction process (Chew *et al.*, 2011).

Extracts rich in phenolic and flavonoid compounds are associated with potential medicinal characteristics. Elevated concentrations of phenolics and flavonoids in extracts govern the pharmaceutical and biological attributes of that particular plant (Mahmoudi *et al.*, 2016). The antioxidant activities and α-glucosidase inhibition by 60% ethanol extract of *H. indica* was probably due to its high phenolic and flavonoid contents (Raza *et al.*, 2018; Khan *et al.*, 2014). Moreover, these functional molecules were also reported to alter the cellular functions in positive way to enhance the system capacity, either to reduce the level of oxidative stress or to improve the repair of impaired physiological machinery under disease condition (Yin *et al.*, 2014; Yamasaki *et al.*, 2010). The significant hypoglycemic potential of 60% ethanol extract owing to the presence of citric acid, procyanidin B3, epicatechin, procyanidin B2, catechin, catechin derivative, procyanidin B1, apigenin-c-hexocide-c-hexocide, kaempferol, kaempferol derivative, quinic acid derivative and gallic acid identified by UHPLC-Q-TOF-MS/MS. The well established antioxidant and antidiabetic activities of these identified compounds were due to phenolic group based structural interaction with ROS and active sites of enzymes (Aadesariya *et al.*, 2017; Zeng *et al.*, 2016; Adefegha *et al.*, 2015; Dewi and Maryani, 2015). The reduction in blood glucose levels of diabetic mice by 60% ethanol extract at high dose are quite comparable with the metformin treated mice group. The physiological alterations are probably due to the interaction of bioactive molecules in extract with the α-glucosidase enzyme in intestine of mice. The anti α-glucosidase activity by natural inhibitors is probably due to enzymatic active site occupation or modification by a particular inhibitor molecule (Martinez-Gonzalez *et al.*, 2017). A recent study compared the *in vitro* hypoglycemic potential of *Cinnamomum zeylanicum* and *Cumin cyminum*. The results indicated the dose dependant inhibition pattern against α-glucosidase enzyme by both spices (Yelaware *et al.*, 2018). Similarly, the antidiabetic effect of *Conocarpus erectus* was evaluated in alloxan induced obese diabetic mice. The extract dose of 450 mg/kg body weight reduced the blood glucose level significantly but the aspect of metabolite profiling was missing in the study (Raza *et al.*, 2018). There may be many reasons behind reduction in blood glucose level of diabetic mice upon consumption of *H. indica* leaf extract including elimination of oxidative stress, repair of

pancreas, enhancement of glucose uptake by cells, as reported by previous investigations (Dra *et al.,* 2018; Rehman *et al.,* 2018). The antioxidant, anti-α-glucosidase and hypoglycemic characteristics of *H. indica* extracts may be attributed due to synergistic mode of action governed by collective contribution of secondary metabolites present in extracts. The *H. indica* may serve as a natural and potent candidate for DM2T management in effective way.

CONCLUSION

The findings of the study confirmed the antioxidant and antidiabetic attributes of *H. indica*. The results of DPPH and total antioxidant power assays reflected the strong antioxidant potential of *H. indica* leaf extracts. The extracts also exhibited notable inhibition of α-glucosidase activity and substantial reduction in blood glucose level of alloxan induced diabetic mice. The study signified the *H. indica* as a promising candidate for diabetes management.

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