Antihyperglycemic effect of *Conocarpus erectus* leaf extract in alloxan-induced diabetic mice

Syed Ali Raza[1], Ayoub Rashid Chaudhary[1], Muhammad Waseem Mumtaz[2]*, Abdul Ghaffar[3]*, Ahmad Adnan[1] and Abdul Waheed[1]

[1]Deapartment of Chemistry, Government College University, Lahore, Pakistan
[2]Department of Chemistry, University of Gujrat, Hafiz Hayyat Campus, Gujrat, Pakistan
[3]Department of Biochemistry, Government College University, Faisalabad, Pakistan

Abstract: Synthetic drugs have widely been helpful in management of diabetes mellitus type 2. However, side effects associated with synthetic drugs serve as an impetus to explore plants as alternate mode of treatment. The hydroethanolic leaf extracts of *Conocarpus erectus* were evaluated for phenolic contents, flavonoid distribution, antioxidant activity and antidiabetic potential. The maximum extract yield, total phenolic and flavonoid contents were exposed by 60% ethanolic extract. The Antioxidant and anti α-glucosidase tendency of 60% ethanolic extract was the most promising and complemented by *in-vivo* antihyperglycemic impact on mice. The findings were substantial regarding suppression of blood glucose levels in alloxan induced diabetic mice establishing the *Conocarpus erectus* as proficient pool of nutraceuticals for diabetes mellitus type 2 management.

Keywords: Freeze drying, phytochemical, antioxidant, antidiabetic, *Conocarpus erectus.*

INTRODUCTION

Oxygen is an integral part of metabolic functions in living systems. The oxygen is often converted into reactive oxygen species (ROS) like singlet oxygen, super oxides, hydroxyl radicals and peroxides as result of various metabolic activities under aerobic environment (Lee & Min, 2004). The ROS are involved in cell signaling but the hazardous impacts also lie with them. The internal antioxidant defense system comprising of multifunctional enzymatic and non-enzymatic entities along with external sources of antioxidants, collectively encounters the ROS to maintain the proper physiology of biological systems (Poljsak *et al.,* 2013; Pizzino *et al.,* 2017). State of oxidative stress develops when the balance between ROS and antioxidant defense becomes out due to multidimensional reasons (Kurutas, 2016). Oxidative stress is associated with wide spectrum of chronic diseases and metabolic disorders including diabetes mellitus by altering the antioxidant enzymes status, lowering of vitamin C level and disturbed glutathione metabolism (Asmat *et al.,* 2016). The diabetes mellitus is a metabolic disorder imprinted by persistent hyperglycemic physiological condition mainly due to low insulin secretion or insulin insensitivity (Patel and Group, 2007; Association, 2014). The diabetes mellitus is under intensive consideration due to its rapid rate of growth and associated side complications. According to estimation, the number of diabetic patients will jump up to 592 million till the year 2035 (Guariguata *et al.,* 2014). Pakistan is also suffering from this medical menace and may be the 7th largest country in world with diabetic population. The disease has become a socioeconomic burden due to its wide and quick expansion in urban as well as rural population of Pakistan (Hussain and Ali, 2016). Diabetes mellitus type 2 (DMT2) is the major type of diabetes and 90-95% diabetic patients come under its coverage (Raz *et al.,* 2013). The influence of modern urbanized life style and unhealthy dietary approaches are the key factors in development of DMT2 and oxidative stress further facilitates the diabetes pathogenesis (Charokopou *et al.,* 2016; Sami *et al.,* 2017). The synthetic drugs and treatments for DMT2 available in market are linked with side complications due to their mode of action at physiological level (Chaudhury *et al.,* 2017). The critical drawbacks of synthetic agents for DMT2 cure serve as an impetus to develop safe and effective therapeutics from alternate sources like plants (Kooti *et al.,* 2016). Plants are natural reserves of antioxidants and nutraceuticals which are effective to combat oxidative stress and to minimize the disease initiation and prolongation (Zaid *et al.,* 2015; Xu et al., 2017). Plants are enriched with α-glucosidase inhibitors exposing their antidiabetic potential with improved physiological status under diseased conditions (Rouzbehan *et al.,* 2017). Main focus to cure diabetes and to minimize its complications moves around management of postprandial hyperglycemia. The α-glucosidase is the key enzyme responsible for the breakdown of complex carbohydrates into simpler sugars. The inhibition of α-glucosidase restricts the hydrolysis of carbohydrates and delays their absorption resulting in controlled blood glucose level (Brunetti and Kalabalik, 2012). The efficient use of α-glucosidase inhibitors can be a

*Corresponding author: e-mails: muhammad.waseem@uog.edu.pk, aghaffaruaf@yahhoo.com

successful therapeutic approach to treat DMT2. Plants gain sound gravity for the DMT2 cure due to presence of natural α-glucosidase inhibitors. The unconditional efficacy, cost-effectiveness, availability, natural and safe nature of nutra-pharmaceuticals emphasized on the need to explore plants for their hidden antidiabetic potential.

Conocarpus erectus (*C. erectus*) belongs to family of *Combretaceae* and commonly exists in tropical and subtropical regions of world (Rosa Galdino Bandeira, 2003). *C. erectus* is well known for its folkloristic medicinal potential including treatment of diabetes (Nascimento *et al.,* 2016). Some scientific confirmations are also presented in support of its curative activities (Abdel-Hameed *et al.,* 2012; Abdel-Hameed *et al.,* 2013). However, the role of *C. erectus* in DMT2 management needed to be explored to proceed for evidence based therapy from natural resource.

MATERIAL AND METHODS

Green extract preparation

Fresh leaves of *C. erectus* were immediately quenched in liquid nitrogen with continuous grinding to bring leaves in powdered form. The powder was freeze dried -68°C for 48 hours on Christ Alpha 1-4 LD (Germany). The powder was subjected to hydroethanol compositions ranging from 100% aqueous to 100% ethanol with multiple intervals of 20 for 48 hours. The resultant mixtures were sonicated on Soniprep 150 ultrasound disintegrator (UK) below 10°C. Each fraction was shaken for 2 hours, filtered on suction assembly and again freeze dried at -68°C for 48 hours. The extract yields were calculated to discriminate extraction solvent systems.

Determination of total phenolic contents

Mixture of 1mL Folin Ciocalteu reagent and 0.1 mg of plant extract was prepared. Then 2mL of 10% Na_2CO_3 were added to the mixture and stayed for two hours. The absorbance of mixture was noted at 765 nm on UV-1700 (Schimadzu Japan) spectrophotometer. Findings were represented as Gallic acid equivalent (GAE) mg/g plant extract (Zengin *et al.,* 2010).

Determination of total flavonoid contents

Initially 100µL of crude plant extracts were dissolved in 2mL of methanol and diluted with 4mL of distilled water. Secondly 0.6mL of 5% $NaNO_2$ solution and 0.6mL of 10% $AlCl_3$ solution were added to previously obtained mixture and stayed for 10 minutes at ambient conditions. At the end 4mL of 1 molar NaOH was added and net volume of 20mL was obtained by adding distilled water. After 25 minutes stay, absorbance was measured at 510 nm and results were represented as Rutin equivalent mg/ g plant extract (Zhishen *et al.,*1999).

Free radical scavenging activity

Free radical scavenging of extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Shahwar and Raza, 2012). Initially 25% DPPH solution in 10mL methanol was made. Each plant extract (50-200 µg) was re-suspended in methanol (1mL) to form extract concentrations of 50-200ppm. After that two hundred µL of each extract solution was then mixed to 1.0mL of 25% DPPH solution. Resultant reaction mixture was allowed to stand for 35 minutes in dark at ambient temperature condition to complete the reaction. The absorbance was measured at 517 nm on UV-1700 (Schimadzu Japan) spectrophotometer. Free radical scavenging potential was finally calculated by using the following formula.

$$\% \text{ DPPH inhibition} = \frac{A - B}{A} \times 100$$

A = Absorbance of blank
B = Absorbance of Sample
BHA was used as standard antioxidant and results were expressed as µg/mL.

In-vitro antidiabetic activity

Inhibitory action of extracts against α-glucosidase was checked to evaluate their antidiabetic potentials. Extracts (10-100µg) were dissolved in 70µL of (50 Mm) phosphate buffer having pH 6.8 and 1unit/mL of α-glucosidase. Incubation of mixture at 37°C for 10 minutes was done and reaction was started by adding 10µL of ρ-nitrophenolglucopyranoside (5mM). After further incubation of 30 minutes, absorbance was measured at 405 nm for released ρ-nitrophenol. All the measurements were made in triplicate. Following formula was used to calculate percentage inhibition (Mediani *et al.,* 2012)

$$\% \text{ inhibition} = \frac{Ab - As}{Ab} \times 100$$

Ab = Absorbance of blank
As = Absorbance of Sample
Acarbose was used as standard reference and results were represented as IC_{50} (µg/mL) values for each extract.

In-vivo antidiabetic activity

A mice model based experiment was designed comprising of 60 number of male mice of 5 to 6 week age having an average body weight of 27.35±1.90g. A prior permission from the ethical committee of GC University Lahore was obtained to run experiment. Mice were shifted to animal house and stayed for ten days acclimatization period at 28°C±2.0°C temperature and relative humidity of 68.75% ±4.5%. Mice were grouped into experimental sets after feeding on normal chow for ten days. The clinical trial was comprised of 10 weeks excluding adaptation period. After eight weeks of ad libitum supply to food and water, 24 hours fasted high fat diet (HFD) group was injected intraperitoneally with alloxan in 10% saline at concentration of 150mg/Kg body weight to induce diabetes. After three days of injection, blood glucose

level (BGL) of alloxan treated mice was measured by glucometer and mice having blood glucose level ≥200 mg/dL were considered as diabetic (Emordi *et al.*, 2016). Diabetic mice were categorized into four groups namely untreated diabetic group (DUG), metformin treated (250 mg/kg body weight) group (MFG), low dose group (LDG, treated with 250 mg/kg body weight of plant extract) and high dose group (HDG) treated with plant extract at rate of 450 mg/kg of body weight (each group comprised of 12 mice). The clinical trial was completed in period of eight week after differentiation into experimental groups.

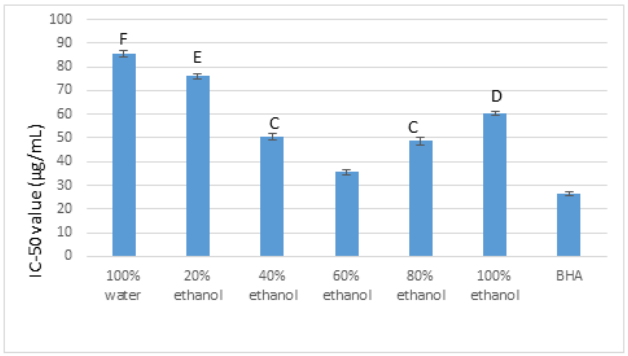

Fig. 1: DPPH assay for antioxidant potential of leaf extracts of C.

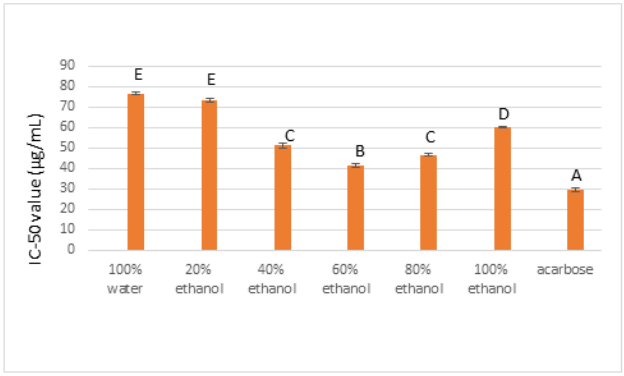

Fig. 2: Anti-α-glucosidase activity of C. erectus leaf extracts

Weight measurement

Body weight (g) measurement was carried out from the start of experiment and variations in weight were noticed weekly. The % weight gain was calculated by following relationship:

$$\% \text{ body weight} = \frac{\text{Weight gain on specific week} \;\; \text{baseline weight}}{\text{Baseline body weight}}$$

Blood glucose level

The blood glucose levels were calculated on weekly basis and were compared to evaluate the efficacy of treatments to manage DMT2. The blood from lateral vein in tail of mice was taken and blood glucose level was measured by glucometer and represented in mg/dL.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was used to evaluate the level of statistical significance among values of experimental findings. Minitab 18 statistical software was utilized for statistical analysis.

RESULTS

Extract yield (%), TPC and TFC

Maximum extract yield of 21.5±0.03[a]% was obtained when extracted with 60% ethanol followed by 18.14± 0.01[b]% with 80% ethanol. The difference in extract yield was might be due to polarity of solvent system and maximum yield with 60% ethanol was supported by some previous studies (Nehete *et al.*, 2010; AL-Zuaidy *et al.*, 2017). Extract yield continuously increased and attained maximum value at 60% ethanol solvent composition, then gradually decreased with further increase in ethanol concentration (table 1).

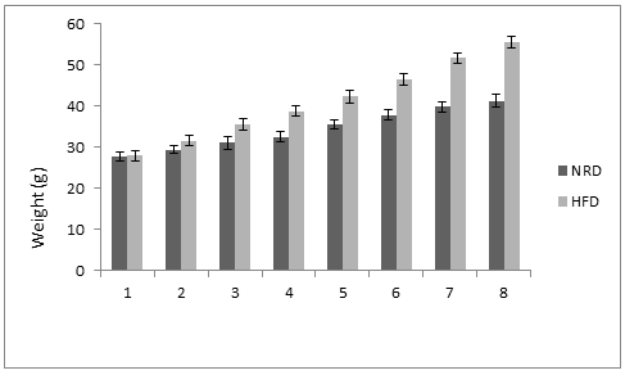

Fig. 3: Comparative weight gains over period of eight weeks, where NRD = Normal Diet Group, HFD = High Fat Diet Group.

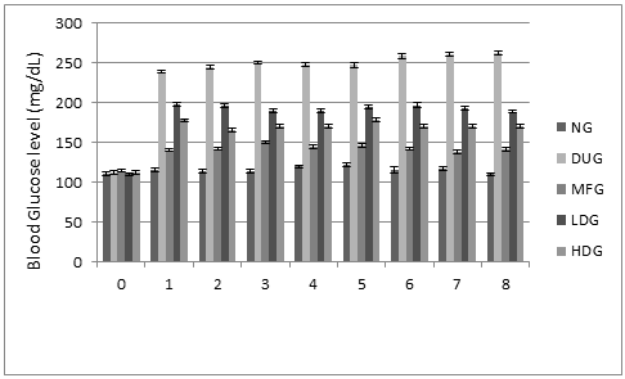

Fig. 4: Blood glucose level of mice under different treatments measured on weekly basis

Results were represented with standard deviation values (±) and significant level was indicated by letter as superscript. PE = Plant extract. Statistical analysis indicated that the solvent composition effect on extract yield was significant.

Table 1: Extract yields, TPC and TFC from leaves of *C. erectus* under different solvent compositions.

Solvent composition	Extract yield (%)	TPC in mg GAE/g PE	TFC in mg Rutin/g PE
0 (100 % water)	11.05 ± 0.04 ^{d}	29.10 ± 1.53 ^{d}	11.25 ± 0.75 ^{d}
20% Ethanol	12.04 ± 0.1 ^{cd}	58.50 ± 1.30 ^{c}	27.30 ± 1.15 ^{c}
40% Ethanol	14.12 ± 0.02 ^{c}	87.50 ± 2.12 ^{b}	40.75 ± 1.25 ^{b}
60% Ethanol	21.5 ± 0.03 ^{a}	117.00 ± 2.05 ^{a}	70.10 ± 2.10 ^{a}
80% Ethanol	18.14 ± 0.01 ^{b}	88.29± 1.95 ^{b}	42.15 ± 1.05 ^{b}
100% Ethanol	14.05 ± 0.02 ^{c}	61.30 ± 1.45^{bc}	30.33 ± 1.11^{bc}

Results were represented with standard deviation values (±) and significant level was indicated by letter as superscript.
PE = Plant extract

Free radical scavenging activity

The DPPH assay was used as conventional tool to determine the antiradical potential of plant extracts. The IC-50 (µg/mL) values were statistically compared to evaluate the significance level of efficiency among hydro-ethanolic leaf extracts (fig. 1).

The findings were statistically significant and 60% ethanolic leaf extract exhibited maximum radical scavenging as compared to other fractions but less effective than the BHA.

In-vitro antidiabetic potential

Antidiabetic potential of *C. erectus* leaf extracts was evaluated by measuring the inhibition of α-glucosidase spectrophotometrically. The results for enzymatic inhibition were statistically significant ($\rho<0.05$) discriminating the fractions and acarbose as antidiabetic agents (fig. 2).

Obesity development

The weights of mice fed with high fat diet (HFD) were compared with normal diet groups (NDG) weekly (fig. 3). An increase of 98.21% was observed for HFD group being much higher than 48.38% (NRD) indicated the substantial obesity development due to diet rich in fat contents. Ad libitum water and food supply was provided to mice during the experiment. Obese mice group was injected with alloxan to induce diabetes successfully.

Blood glucose levels (BGL)

Impact of 60% ethanolic leaf extract on blood glucose concentrations at two doses (250 and 450 mg/kg body weight) was measured and compared with NG and MFG.

DISCUSSION

Findings revealed that TPC were also influenced by the polarity differences among the designed solvent composition. Maximum TPC value of 117±2.05^{a} mg GAE/g was obtained from extract obtained by 60% ethanol. Similarly, the highest TFC were also extracted in 60% ethanol with the value of 70.10± 2.10^{a}. The values of TPC and TFC harmonized the extract yield generated by combinational solvent approach for extraction. This emphasized that the phenolic compounds extraction affected by solvent composition was highly important due to biological significance of phenolics especially as antioxidants (Shahzadi *et al.,* 2011).

The extracts reduced stable nitrogen centered DPPH radical to different extents indicating the reduction potential. The spectroscopic measurements were based upon change in color intensity due to shift from violet to yellow, a bathochromic shift (Dehpour *et al.,* 2009). Reduction potential was the measure of antioxidant activity.

The antiradical potential of 60% ethanolic fraction was probably due to the comparatively high TPC and TFC (Baba and Malik, 2015). The strong antioxidant activity by the leaf extracts of *C. erectus* made this plant a natural enriched pool of functional agents.

The functionalities associated with the 60% ethanolic extract were might be due to the high presentation of TPC and TFC as compared with other fractions. The aqueous fraction was the least effective to restrict the enzymatic activity. The 40% and 80% ethanolic extracts were statistically non-significant opted as higher ones after 60% extract. However, no extract could meet the acarbose to inhibit the α-glucosidase as acarbose was associated with minimum IC-50 value. The recent study also established the exceptionally high antidiabetic potential of acarbose when used as standard antidiabetic reference agent (AL-Zuaidy *et al.,* 2016; Safithri and Sari, 2016). The obesity and diabetes are closely linked due to physiological alternations caused by oxidative stress in system (Walton, 2017).

CONCLUSION

The 60% ethanol was proved as best choice for enhanced extraction of TPC and TFC from leaves of *C. erectus*. The extract was efficient α-glucosidase inhibitor and suppressed glycemic concentrations in blood of diabetic mice. The leaves of *C. erectus* may serve as natural source of bioactive entities for nutraceutical development to combat DMT2. The findings revealed the efficacy of plant extract to control blood glucose level. The HDG

exhibited sufficient reduction in BGL when compared with NG but less effective as was the case with MFG (fig. 4). Both extract concentrations restricted the rise in BGL above 200 mg/dL, the threshold level but HDG was more promising. The proficient efficacy of leaf extracts to suppress BGL proved as novel tool to treat DMT2 in alternate mode to get rid of synthetic drugs. The antihyperglycemic attributes of 60% ethanolic extract were probably due to comparatively higher amounts of TPC and TFC, the valuable chemical entities to treat chronic ailments (Asgar, 2013; Vinayagam and Xu, 2015). The role of bioactive ingredients of plants may interfere with the redox reactions in living system to minimize the oxidative stress and pathogenesis (Al-Waili *et al.,* 2017). The heterogeneous chronicity of DMT2 can be overcome by considering the integrative and alternative mode of treatment with minimum side complications and *C. erectus* may be a workable choice.

REFERENCES

Abdel-Hameed ESS, Bazaid SA and Sabra ANA (2013). Protective effect of *Conocarpus erectus* extracts on CCl4-induced chronic liver injury in Mice. *Glob J. Pharmacol.,* **7**(1): 52-60.

Abdel-Hameed ESS, Bazaid SA, Shohayeb MM and El-Sayed MM (2012). Phytochemical studies and evaluation of antioxidant, anticancer and antimicrobial properties of *Conocarpus erectus* L. growing in Taif, Saudi Arabia. *European J. Med. Plants.,* **2**(2): 74-92.

Al-Waili N, Al-Waili H, Al-Waili T and Salom K. (2017). Natural antioxidants in the treatment and prevention of diabetic nephropathy; a potential approach that warrants clinical trials. *Redox Rep.,* **22**(3): 99-118.

AL-Zuaidy MH, Mumtaz MW, Hamid AA, Ismail A, Mohamed S and Razis AFA (2017). Biochemical characterization and 1H NMR based metabolomics revealed *Melicope lunu-ankenda* leaf extract a potent anti-diabetic agent in rats. *BMC complement altern med.,* **17**(1): 359.

AL-Zuaidy MH, Hamid AA, Ismail A, Mohamed S, Razis AF, Mumtaz MW and Salleh Z (2016). Potent antidiabetic activity and metabolite profiling of *Melicope lunu-ankenda* leaves. *J. Food Sci.,* **81**(5): C1080-1090.

Asgar MA (2013). Anti-diabetic potential of phenolic compounds: A review. *Int. J. Food Prop.,* **16**(1): 91-103.

Asmat U, Abad K and Ismail K (2016). Diabetes mellitus and oxidative stress a concise review. *Saudi Pharm J.,* **24**(5): 547-553.

Association AD (2014). Diagnosis and classification of diabetes mellitus. *Diabetes Care.,* **37**(1): S81-S90.

Baba SA and Malik SA (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii Blume*. *J Taibah Univ Sci.,* **9**(4): 449-454.

Brunetti L and Kalabalik J (2012). Management of type-2 diabetes mellitus in adults: Focus on individualizing non-insulin therapies. *PT.,* **37**(12): 687-96.

Charokopou M, Sabater F, Townsend R, Roudaut M, McEwan P and Verheggen B (2016). Methods applied in cost-effectiveness models for treatment strategies in type 2 diabetes mellitus and their use in Health Technology Assessments: A systematic review of the literature from 2008 to 2013. *Curr. Med. Res. Opin.,* **32**(2): 207-218.

Chaudhury A, Duvoor C, Dendi VSR, Kraleti S, Chada A and Ravilla R (2017). Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. *Front Endocrinol.,* **8**(6). https://doi.org/10.3389/fendo.2017.00006

Dehpour AA, Ebrahimzadeh MA, Fazel NS and Mohammad NS (2009). Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. *Grasas aceites.,* **60**(4): 405-412.

Emordi JE, Agbaje EO, Oreagba IA and Iribhogbe OI (2016). Antidiabetic and hypolipidemic activities of hydroethanolic root extract of *Uvaria chamae* in streptozotocin induced diabetic albino rats. *BMC complement Altern Med.,* **16**(1): 468.

Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U and Shaw JE (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract.,* **103**(2): 137-149.

Hussain A and Ali I (2016). Diabetes mellitus in Pakistan: A major public health concern. *Arch Pharm Pract.,* **7**(1): 30-32.

Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D and Asadi-Samani M (2016). The role of medicinal plants in the treatment of diabetes: A systematic review. *Electronic physician.,* **8**(1): 1832-1842.

Kurutas EB (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. *Nutr. J.,* **15**(1): 71.

Lee J, Koo N and Min DB (2004). Reactive oxygen species, aging and antioxidative nutraceuticals. *Compr Rev. Food Sci. Food Saf .,* **3**(1): 21-33.

Mediani A, Abas F, Ping TC, Khatib A and Lajis NH (2012). Influence of growth stage and season on the antioxidant constituents of *cosmos caudatus*. *Plant. Food. Hum. Nutr*., **67**: 344-50.

Nascimento DK, Souza IA, Oliveira AFD, Barbosa MO, Santana MA and Pereira Junior DF (2016). Phytochemical screening and acute toxicity of aqueous extract of leaves of *Conocarpus erectus* linnaeus in swiss albino mice. *An. Acad. Bras. Ciênc.,* **88**(3): 1431-1437.

Nehete J, Bhatia M and Narkhede M (2010). *In vitro* evaluation of antioxidant activity and phenolic content of *Costus speciosus* (Koen) JE Sm. *Iran J. Pharm Res.,* **9**(3): 271-277.

Patel A, Group AC, MacMahon S, Chalmers J, Neal B, Woodward M, Billot L, Harrap S, Poulter N, Marre M, Cooper M, Glasziou P, Grobbee DE, Hamet P, Heller S, Liu LS, Mancia G, Mogensen CE, Pan CY, Rodgers A and Williams B (2007). Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): A randomised controlled trial. *Lancet.,* **370**(9590): 829-840.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F and Arcoraci V (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxid med cell longev.,* 8416763.10.1155/2017/8416763

Poljsak B, Šuput D and Milisav I (2013). Achieving the balance between ROS and antioxidants: When to use the synthetic antioxidants. *Oxid med cell longev.*,http://dx.doi.org/10.1155/2013/956792

Raz I, Riddle MC, Rosenstock J, Buse JB, Inzucchi SE and Home PD et al. (2013). Personalized management of hyperglycemia in type 2 diabetes: Reflections from a Diabetes Care Editors' Expert Forum. *Diabetes Care.*,**36**(6): 1779-1788.

Rosa Galdino Bandeira A (2003). Estudo Fitoquímico e Atividade Biológica de Conocarpus erectus L.(Mangue botão). http://repositorio.ufpe.br/handle/123456789/1837

Rouzbehan S, Moein S, Homaei A and Moein MR (2017). Kinetics of α-glucosidase inhibition by different fractions of three species of *Labiatae* extracts: A new diabetes treatment model. *Pharm. Biol.,* **55**(1): 1483-1488.

Safithri M and Sari YP (2016). Inhibition of α-glucosidase activity by ethanolic extract of *Melia azedarach* L. leaves. Paper presented at the IOP Conf. *Ser: Earth Environ Sci.,* **31**(1): 012025

Sami W, Ansari T, Butt NS and Ab Hamid MR (2017). Effect of diet on type 2 diabetes mellitus: A review. *Int J Health Sci (Qassim).,* **11**(2): 65-71.

Shahwar D and Raza MA (2012). Antioxidant potential of phenolic extracts of *Mimusops elengi*. *Asian Pac. J. Trop Biomed.,* **2**(7): 547-550.

Shahzadi T, Abbasi MA, Riaz T, Rehman A, Siddiqui SZ and Ajaib M (2011). *Caryopteris odorata*: A rich source of antioxidants for protection against chronic diseases and food products. *J. Chil. Chem. Soc.,* **56**(2): 678-681.

Vinayagam R and Xu B (2015). Antidiabetic properties of dietary flavonoids: A cellular mechanism review. *Nutr Metab.*,**12**: 60.10.1186/s12986-015-0057-7

Walton EL (2017). Oxidative stress and diabetes: Glucose response in the cROSsfire. *Bimed J.,* **40**(5): 241-244.

Xu DP, Li Y, Meng X, Zhou T, Zhou Y and Zheng J (2017). Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *Int. J. Mol. Sci.,* **18**(1): 96.10.3390/ijms18010096

Zaid H, Saad B, Mahdi AA, Tamrakar AK, Haddad PS and Afifi FU (2015). Medicinal plants and natural active compounds for diabetes and/or obesity treatment. *Evid. Based Complement. Alternat. Med.,*10.1155/2016/2965214

Zengin G, Cakmak YS, Guler GO and Aktumsek A (2010). *In vitro* antioxidant capacities and fatty acid compositions of three *Centaurea* species collected from Central Anatolia region of Turkey. *Food Chem Toxicol.,* **48**(10): 2638-2641.

Zhishen J, Mengcheng T and Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.,* **64**(4): 555-559.