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Cytoprotective, antihyperglycemic and phytochemical properties of *Cocos nucifera* (L.) inflorescence

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ABSTRACT

Objective: To analyze the cytoprotective and antidiabetic activities as well as phytochemical composition of the immature inflorescence of *Cocos nucifera* belonging to the Arecaceae Family. **Methods:** The phytochemical screening of inflorescence was done to determine the major constituents present in *Cocos nucifera* inflorescence. The free radical scavenging potential of inflorescence extracts were evaluated using *in vitro* radical scavenging assay models. **Results:** The phytochemical analyses on inflorescence showed the presence of phenolic compounds, flavonoids, resins and alkaloids. The macronutrient analyses, on the other hand, showed the presence of carbohydrate, proteins and fibers. Administration of the methanol extract of coconut inflorescence to the diabetic rats showed dose dependent reduction in hyperglycemia. The cytoprotective property of coconut inflorescence was evidenced from the acute toxicological evaluation. The levels of serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were significantly decreased in the diabetic rats treated with inflorescence when compared with the diabetic control rats. **Conclusion:** The results obtained from the present study apparently proved the non-toxic nature and the cytoprotective and antihyperglycemic properties of coconut inflorescence.

1. Introduction

Plants have been used as a source of traditional medicine for years to treat various diseases and conditions. Many of these medicinal plants are also excellent sources of phytochemicals, many of which have potent antioxidant activities[1]. They reduce the risk of various diseases due to the presence of these antioxidant compounds[2]. Most of these phytochemicals are produced via secondary metabolism in relatively small amounts[3]. A great number of plants worldwide have proved to present a strong antioxidant activity and a powerful scavenger activity against free radicals[4-5]. Plant antioxidants are composed of a broad variety of different substances like ascorbic acid, tocopherols, polyphenolic compounds, or flavonoids. The antioxidant activities of phenolics are mainly due to their redox properties that allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential[6].

There is an increased evidence for the participation of free radicals in the aetiology of various diseases like diabetes, cancer, cardiovascular diseases, and aging[7]. A wide range of antioxidants from both natural and synthetic origin have been proposed for use in the treatment of various human diseases including diabetes. Diabetes mellitus (DM) is a chronic disease caused by insufficient production of insulin by pancreatic islets and a decrease in absorption of glucose by the cells in the human system, causing increase in the concentration of glucose in blood. DM is rapidly increasing in developing countries of the world. With this increasing incidences worldwide, DM will likely continue to be a leading cause of morbidity and mortality for the foreseeable future[8]. There has been a very urgent need to discover long term effective treatment for this disease and current interests are being geared towards natural medicines, which account for 80% of the world's primary health care needs according to the WHO[9].

Coconut (*Cocos nucifera* L.) is a large palm belonging to

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the family Arecaceae or Palmae. Different coconut products like coconut oil, kernel and water are used as culinary items and as natural remedy for curing some ailments in tropical regions of the world. Because of its versatile properties, coconut palm is considered very auspicious in Indian system of medicine and culture. In *Cocos nucifera*, the flowers and the flower bearing branched stalk are collectively called inflorescence, which is covered by a spathe. In immature inflorescence, the male and female florets lie very close to the peduncle and the whole is so tightly packed. Thus, the individual florets cannot be distinguished. In Indian folk medicine, the fresh juice of *Cocos nucifera* inflorescence (CnI) is used in treating dyspepsia, diarrhea, dysentery, diabetes, haemoptysis and strangury. In most of these cases, scientific proof in terms of modern medicine is lacking. However, nowadays, it is essential to provide scientific evidence to justify the use of a plant or its active principles[10]. In this context, we reported the protective effects of CnI in experimental diabetes and its associated complications[11-12]. Based on the above facts, the present study was planned to evaluate the cytoprotective property and phytochemical analysis of CnI in *in vitro* and *in vivo* experimental models.

2. Materials and methods

2.1. Chemicals

DPPH (1,1-diphenyl-2-picryl-hydrazil) and streptozotocin (STZ) were purchased from Sigma Chemicals, St.Louis, USA. Kit for insulin was purchased from SPI-BIO, France. Kits for glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) estimations were purchased from Agappe Diagnostics, Thane, India. All other chemicals used were of the highest analytical grade.

2.2. Plant material

Unopened immature inflorescence of coconut palm (West Coast Tall variety) was harvested from Kerala University campus. The plant material was identified at Department of Botany in the University and a sample specimen (KUBH 5795) was deposited in the department herbarium. The inflorescence was then removed from spathe and pulverized well using an electric laboratory blender. It was then dried at 55 ℃ in a hot air oven, weighed and used for phytochemical and macronutrient analyses.

2.3. Preparation of CnI extracts

CnI was extracted with different solvents (methanol and ethanol). To prepare CnI extracts, 25 g aliquots of oven dried powdered CnI were stirred with 80% (v/v) methanol and 95% (v/v) ethanol respectively and soaked overnight in refrigerator. Two extraction replicates of each solvent were prepared for each CnI sample. The suspensions were filtered and the residues were re-extracted twice. These different solvent extracts of CnI were pooled and evaporated in a rotary flash evaporator (Heidolph Laborota-4000) at 45-50 ℃ under reduced pressure and lyophilized. The yields of freeze dried materials were 6.29 and 5.62 g respectively for methanol and ethanol extracts. The extracts were stored at 0 ℃ until used.

2.4. In vitro studies

2.4.1. Qualitative analysis

A systematic study of a crude plant material embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. Thus, CnI extracts were subjected to various qualitative tests for the identification of different phytochemical constituents[13].

2.4.2. Quantitative analysis

Whole dried powdered CnI was used for the following quantitative estimations. Total crude proteins were estimated by Lowry's method[14]. Total fat content was estimated using petroleum ether method[15]. Crude fiber was estimated as neutral detergent fiber as described earlier[16]. Anthrone method was employed for estimating total carbohydrate content[17]. Total polyphenol content was determined using Folin-Ciocalteu method[18]. Analysis of total amino acids present in CnI was carried out using HPLC and the results are expressed as area %[19].

2.4.3. Free radical scavenging activities

The free radical scavenging activity of the methanol and ethanol extracts of CnI were measured by DPPH method[20]. Superoxide anion scavenging activity of CnI extracts were assayed using a nonenzymatic phenazine methosulphate nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH and oxygen[21].

2.5. In vivo studies

2.5.1. Animals and experimental design

Male Sprague Dawley rats (150-200 g body weight) bred in our department animal house was used for the study. The animals were housed individually in polypropylene cages in a room maintained at (25±5) ℃ with a 12 h light and 12 h dark cycle and were divided into five groups of six rats each and fed as follows: Group 1, Normal control; Group 2, Diabetic control; Group 3, Diabetic + CnI methanol extract [100 mg/(kg·d)]; Group 4, Diabetic + CnI methanol extract

[200 mg/(kg·d)]; Group 5, Diabetic + CnI methanol extract [400 mg/(kg·d)].

This experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and all the animal care and procedures were done in compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Diabetes was induced in the rats of the group 2, 3, 4 and 5 by injection with a single dose of STZ (45 mg/kg body weight, dissolved in 0.1 mol/L citrate buffer, pH 4.5) intraperitoneally after overnight fasting. The rats were then kept on 50 g/L glucose solution for the next 24 h to prevent drug induced hypoglycemic phase. The fasting blood glucose levels of all the injected rats were checked after 72 h. Rats with a blood glucose level ≥200 mg/dL were considered to be diabetic. After 45 days of experimental period, animals were fasted overnight and sacrificed by intraperitoneal injection of thiopentone sodium (>40 mg/kg body weight). Blood was collected and serum was separated for biochemical and toxicological estimations.

2.5.2. Biochemical estimations

Serum glucose level was estimated using a commercial kit based on glucose oxidase/peroxidase enzymatic method[22]. Serum insulin level was estimated by enzyme immune assay (EIA) method using a commercial insulin kit (SPI-BIO, France)[23].

2.5.3. Toxicological evaluations

ALT and AST activities in serum were determined by the method of Reitman and Frankel[24]. Serum ALP activity was determined according to the method of King and Armstrong[25].

2.6. Statistical analysis

The data were statistically evaluated with Statistical Package for Social Sciences (SPSS) version 17. (SPSS Inc., Chicago, IL, USA). Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Duncan's post hoc multiple variance test. $P<0.05$ was considered to indicate statistical significance. The results are expressed as mean±S.D for six rats in each group.

3. Results

3.1. In vitro qualitative and quantitative phytochemical analyses

Qualitative analysis of the CnI methanol and ethanol extracts revealed the presence of proteins, carbohydrates, phenolic compounds, flavonoids, alkaloids, tannins and resins (Table 1). Quantitative estimation of the dried powdered CnI revealed the presence of proteins, carbohydrates, fats, crude fiber and polyphenols. The results of HPLC analysis of amino acids present in CnI are shown in Figure 1. From the data, it was observed that CnI was rich in simple amino acids like glycine, threonine and serine, while arginine, leucine, isoleucine and aspartate were present at moderate concentrations.

Table 1
Qualitative phytochemical analysis of CnI extracts.

Phytochemical	Methanol extract	Ethanol extract
Alkaloids		
Mayer's test	+	++
Wagner's test	+	++
Picric acid test	+	++
Hagner's test	+	++
Marquis test	+	++
Flavonoids		
Ammonium test	+++	−
Aluminium chloride test	−	−
NaOH test	+++	+++
HCl-Mg ribbon test	−	−
Saponins		
Emulsion test	−	−
Frothing test	−	−
Resins		
Precipitation test	−	−
Color test	+++	+++
Tannins		
Ferric chloride test	++	+
Lead acetate test	+++	+
Steroids & terpenoids		
Sulphuric acid test	++	+
Terpenoids test	−	−
Acidic components		
Litmus test	++	++
Anthraquinones		
HCl-chloroform test	−	++
Borntragers test	−	+
Carbohydrates		
Moilsch's test	+++	++
Protein		
Ninhydrin test	+	−
Biurete test	+	−
Fat and oils		
Translucency test	−	−
Saponification test	−	−
Chlorogenic acid		
Aqueous ammonia test	+++	+++

Inferences are means of triplicate determination. +++ Present; ++ Moderately present; + Slightly present; − Absent.

3.2. Effect of CnI extracts on DPPH and superoxide anion radical scavenging

The methanol extract of CnI showed an efficient scavenging of DPPH and superoxide anion radicals, compared to the ethanol extract. At 80 μg/mL concentration, there was 63%

inhibition of DPPH by the methanol extract, and 61% by the ethanol extract was observed. The IC_{50} values were found to be 40.5 and 46.1 μg/mL respectively for the methanol and ethanol extracts (Figure 2). In the case of superoxide radicals, the percentage inhibitions were 71% and 63% respectively at a concentration of 200 μg/mL with an IC_{50} of 120.0 and 129.5 μg/mL (Figure 3). IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH and superoxide anion radicals. A lower value of IC_{50} indicates a higher antioxidant activity. Both these experiments were repeated three times. Butylated hydroxytoluene (BHT) was used as the positive control in these assays.

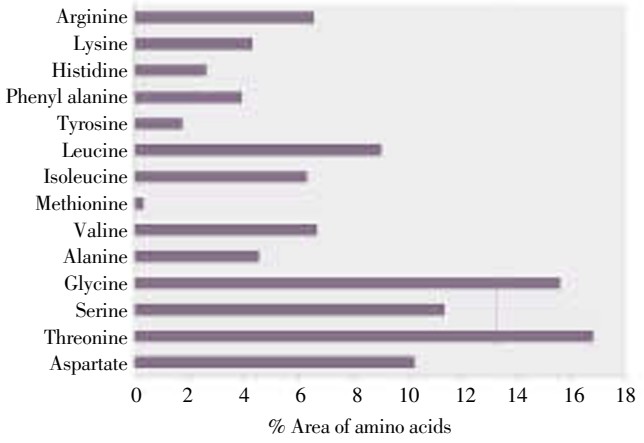

Figure 1. HPLC analysis of amino acids present in *Cocos nucifera* inflorescence.

3.3. Effect of CnI extract on biochemical indicators

Administration of STZ significantly elevated the levels of fasting blood glucose (Table 2) of the diabetic control rats, compared to those of the normal animals. Administration of the methanol extracts of CnI to diabetic rats showed significant reduction in the fasting glucose levels compared to that in the diabetic control. The extract at the dose of 200 mg/kg body weight was found to possess better antihyperglycemic effect, compared to that at the other doses. Serum insulin levels were found to be decreased in the diabetic control rats. The CnI methanol extract significantly increased insulin levels in a dose dependant manner in the diabetic rats. Serum insulin levels of rats in the Group 4 were higher than those of rats in the Group 3 and 5 (Table 2).

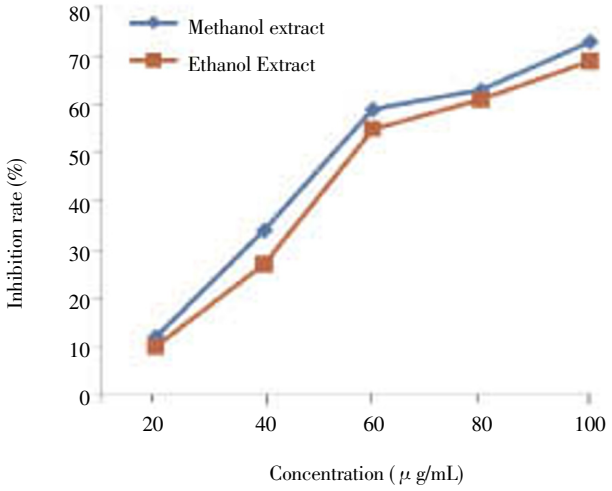

Figure 2. DPPH radical scavenging assay of *Cocos nucifera* inflorescence extracts.
Values are means of triplicate determination. IC_{50} values of the methanol extract and ethanol extract were 40.5 and 46.1 μg/mL, respectively.

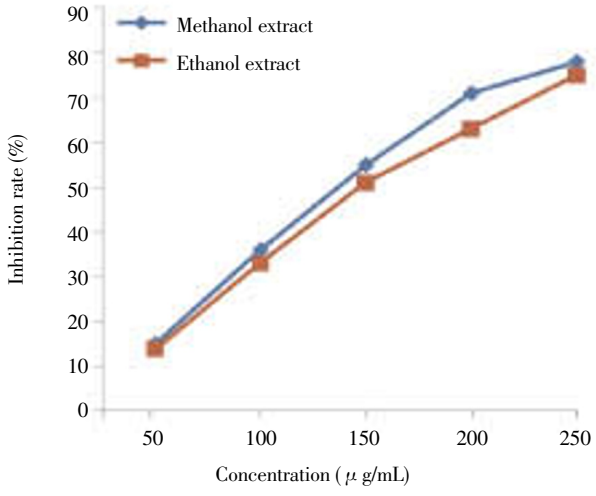

Figure 3. Superoxide anion scavenging assay of *Cocos nucifera* inflorescence extracts.
Values are means of triplicate determination. IC_{50} values of the methanol extract and ethanol extract were 120.0 and 129.5 μg/mL, respectively.

Table 2
Effects of CnI methanol extracts on serum biochemical and toxicological indicators.

Group	Glucose level (mg/dL)	Insulin level (ng/mL)	AST (IU/L)	ALT (IU/L)	ALP (Katal/L)
Group 1	74.45±6.50	3.75±0.36	78.19±6.90	28.10±2.90	22.14±2.30
Group 2	216.23±18.50*	0.97±0.04*	132.38±11.90*	58.35±4.90*	48.45±4.50*
Group 3	112.61±8.70	2.10±0.15	110.14±8.10	42.68±4.20	35.52±3.70
Group 4	107.91±7.90#	2.50±0.20#	102.57±6.70#	36.04±3.80#	31.56±3.20#
Group 5	130.22±11.40	1.93±0.12	114.41±8.90	48.70±4.60	38.57±4.00

Values are mean±SD of six rats. *$P<0.05$ compared to normal control; #$P<0.05$ compared to diabetic control. Group 1, Normal control; Group 2, Diabetic control; Group 3, Diabetic + CnI methanol extract [100 mg/(kg.d)]; Group 4, Diabetic + CnI methanol extract [200 mg/(kg.d)]; Group 5, Diabetic + CnI methanol extract [400 mg/(kg.d)]. 1 Katal=6×10^7 IU.

3.4. Effect of CnI extract on toxicological markers

Administration with STZ significantly elevated the activities of serum ALT and AST of the diabetic control rats, compared to those of the normal rats. In the present study, treatment with the CnI methanol extract at the dose of 200 mg/kg body weight significantly reversed these changes compared to that at other doses, indicating better cytoprotection (Table 2). The activity of serum ALP of the control and STZ-diabetic rats is shown in Table 2. The activity of this enzyme was found to be significantly increased in the serum of the diabetic rats compared to that of the normal control. Oral administration of the CnI methanol extract for a period of 45 days resulted in the near normalization of serum ALP activity of the diabetic rats.

4. Discussion

This study dealed with the cytoprotective, antihyperglycemic and phytochemical evaluation of the young inflorescence of *Cocos nucifera*. The phytochemical analyses of the young inflorescence of coconut palm showed the presence of phenolic acids, flavonoids and resins at high concentrations as indicated by the intensity of the colored solution and precipitates formed on detection. Alkaloids, acidic compounds, and tannins were present at moderate concentrations. Steroids and saponins had the least concentrations. The macronutrient analyses showed the presence of carbohydrates at a high concentration and proteins at a moderate concentration. However, fats and oils were present at the least concentrations. A smaller quantity of some phytochemicals were present in one of the extract, while a higher quantity were present in the other extract. This can be attributed to their volatility as they may have been evaporated during the concentration of extract by a rotary evaporator or heating in a water bath. From this study, it is clear that the presence of polyphenols and flavonoids though at varying concentrations contributes the antioxidant property of CnI. Phytochemicals have received a great deal of attention because of their antioxidant activity. Antioxidants help to neutralize free radicals, which are unstable molecules that are linked to the development of a number of degenerative diseases like diabetes and cardiovascular diseases[26-27].

In this study, DPPH scavenging activity of CnI methanol fraction indicated high antioxidant potential. Other studies carried out on DPPH radical scavenging of phytocompounds have shown similar results[28]. Because of their high reactivity, most free radicals react rapidly with oxidizable substrates. Therefore, stable model free radicals like DPPH which can be used as indicators for radical scavenging abilities of biological samples are widely used[29]. Superoxide radical (O_2^-) is generated by four electron reduction of molecular oxygen into water. This radical is also formed in aerobic cells because of electron leakage from the electron transport chain[30]. In the present study, the two extracts had a scavenging activity on the superoxide radicals in a dose dependent manner. The methanol extract had stronger superoxide radical scavenging activity than the ethanol extract. These findings are in agreement with the previous reports[31]. The scavenging of superoxide radical by the CnI extracts may be attributed to their various phenolic constituents.

The present study also described the dose dependent modulation of glucose homeostasis by the 80% (v/v) methanol extract of CnI on STZ induced experimental diabetes. STZ is a broad-spectrum antibiotic from *Streptomyces achromogenes*. Since the finding that STZ possesses diabetogenic properties mediated by pancreatic β-cell destruction, this compound has been widely used to induce diabetes in experimental animals. Evidence has suggested that STZ induces oxidative stress. Once STZ enters inside the cell, it is able to spontaneously decompose to form an isocyanate compound and a methyldiazohydroxide. The DNA damage of β-cells of pancreas is mainly by alkylation with carbonium ion produced by these compounds[32]. Oral administration of the CnI methanol extract after STZ treatment resulted in lower serum glucose levels and increased serum insulin levels as compared with the rats administered STZ alone. The results obtained in the current study are in accordance with those of other investigations using different plant extracts[33-34].

In diabetes condition, an increase in serum ALP has been observed, while a decrease in the activity of ALP was observed during treatment with insulin[35]. In this study, treatment with the CnI methanol extract brought down such elevated levels of ALP significantly in the STZ diabetic animals. Transaminases are an important class of enzymes linking carbohydrates and amino acid metabolism which can establish a relation between the intermediates of tricarboxylic acid cycle[36]. Serum ALT and AST are widely used as markers for acute and chronic cellular damage and these are elevated in pathological conditions like diabetes. Elevated levels of ALT and AST were reported in animals treated with toxic plant derived products[37]. Oral administration of the CnI methanol extracts to diabetic rats reduced these parameters significantly in a dose dependant

manner. Therefore, it may be clearly evidenced that this extract is non-toxic to the rodents.

Thus, it can be concluded that the phytochemical analyses of the whole and the extracts of CnI revealed the presence of polyphenols, phenolic acids, flavonoids, resins, proteins, dietary fibers and amino acids. We found that the CnI extracts decreased the *in vitro* formation of free radicals. Hence, the present investigation suggested that the methanolic extract of CnI possessed a very good antioxidant and free radical scavenging potential. Besides this, the methanolic extract of CnI at a dose of 200 mg/kg body weight was found to be more effective in controlling hyperglycemia induced by STZ and it may be mainly due to the presence of polyphenols and dietary fibers and partly to the presence of amino acids like arginine, leucine and isoleucine, which have known antidiabetic properties[38-40]. Moreover, the present study clearly proved the non-toxic nature and the cytoprotective property of CnI. In this regard, CnI may be developed as a promising natural antidiabetic medication with no cytotoxic effects and this should be further confirmed by clinical trials.

Conflict of interest statement

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

[1] Liu H, Qiu N, Ding H, Yao R. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. *Food Res Int* 2008; **41**: 363-370.

[2] Hark L, Deen D. *Nutrition for Life*. London: Dorling Kindersley Publishers; 2006, p. 213

[3] Hasler CM. Functional foods: Their role in disease prevention and health promotion. *Food Technol* 1998; **52**: 63-70.

[4] Chon SU, Heo BG, Park YS, Kim DK, Gorinstein S. Total phenolic level, antioxidant activities and cytotoxicity of young sprouts of some traditional Korean salad plants. *Plant Foods Hum Nutr* 2009; **62**: 167-179.

[5] Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Alvarez JA. Spices as functional foods. *Crit Rev Food Sci Nutr* 2011; **51**(1): 13-28.

[6] Fernandez-Lopez J, Aleson-Carbonell L, Sendra E, Sayas-Barberá E, Angel Pérez-Alvarez J. Dietary polyphenols as functional ingredients: relevance in citrus fruits. *Tree Forestry Sci Biotech* 2009; **2**(Special Issue 1): 120-126.

[7] Beckman KB, Ames BN. The free radical theory of aging matures. *Physiological Rev* 1998; **78**: 547-581.

[8] Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, et al. *Harrison's principles of internal medicine*. 15th ed. New York: McGraw Hill Medical; 2001.

[9] Sharma S, Chatuurvedi M, Edwin E, Shukla S, Sagrawat H. Evaluation of the phytochemicals and antidiabetic activity of *Ficus bengalensis*. *Int J Diab Dev Ctries* 2007; **27**(2): 56-59.

[10] Singh RP, Padmavathi B, Rao AR. Modulatory influence of *Adhatoda vesica* (*Justica adhatoda*) leaf extract on the enzyme of xenobiotic metabolism, antioxidant status and lipid peroxidation in mice. *Mol Cel Biochem* 2000; **213**: 99-109.

[11] Renjith RS, Rajamohan T. Protective and curative effects of *Cocos nucifera* inflorescence on alloxan-induced pancreatic cytotoxicity in rats. *Indian J Pharmacol* 2012; **44**: 555-559.

[12] Renjith RS, Rajamohan T. Young inflorescence of *Cocos nucifera* contributes to improvement of glucose homeostasis and antioxidant status in diabetic rats. *Int J Diabetes Dev Ctries* 2012; **32**: 193-198.

[13] Harborne JB. *Phytochemical methods*. London: Chapman and Hall; 1998, p. 60-68.

[14] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275.

[15] AOAC International. *Official methods of analysis*. 18th ed. Arlington: Assoc. Off. Anal. Chem; 2006.

[16] Kurup PA, Jayaumari N, Indira M, Kurup GM, Vargheese T, Mathew A, et al. Composition, intake, and excretion of fiber constituents. *Am J Clin Nutr* 1984; **40**: 942-946.

[17] Hedge JE, Hofreiter BT. *Carbohydrate chemistry*. 17th ed. New York: Academic Press; 1962.

[18] Amensour M, Sendra E, Abrini J, Bouhdid S, Pérez-Alvarez JA, Fernández-López J. Total phenolic content and antioxidant activity of Myrtle (*Myrtus communis*) extracts. *Nat Prod Comm* 2009; **4**: 819-824.

[19] Ishida Y, Fujita T, Arai K. New detection and separation method for amino acid by high performance liquid chromatography. *J Chromatogr* 1981; **204**: 143-148.

[20] Sanchez-Moreno C. Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci*

Technol Int 2002; **8**: 121-137.

[21]Liu F, Ooi VEC, Chang ST. Free radical scavenging activity of mushroom polysaccharide extract. *Life Sci* 1997; **60**: 763-771.

[22]Lott J A, Turner K. Evaluation of Trinder's glucose oxidase method for measuring glucose in serum and urine. *Clin Chem* 1975; **21**: 1754-1760.

[23]Grassi J, Pradelles PH. Compounds labelled by the acetylcholinesterase of *Electrophorus electricus*. Its preparation process and its use as a tracer or marquer in enzymo-immunological determinations. US patent 1,047,330. September 10, 1991.

[24]Reitman S, Frankel S. A colourimetric method for the determination of serum glutamic oxaloacetic acid and glutamine pyruvate transaminase. *Am J Clin Pathol* 1957; **28**: 56-63.

[25]King EJ, Armstrong AR. A convenient method for determining serum and bile phosphatase activity. *Can Med Assoc J* 1934; **31**(4): 376-381.

[26]Folts JD. Potential health benefits from the flavanoids in grape products in vascular disease. *Adv Exp Med Biol* 2002; **505**: 95-111.

[27]Leborgne L, Mazière JC, Mazière C, Andrejak M. Oxidative stress, atherogenesis and cardiovascular risk factors. *Arch Mal Coeur Vaiss* 2000; **95**(9): 805-814.

[28]Ramamurthy CH, Kumar MS, Suyavaran VS, Mareeswaran R, Thirunavukkarasu C. Evaluation of antioxidant, radical scavenging activity and polyphenolics profile in *Solanum torvum* L. fruits. *J Food Sci* 2012; **77**(8): 907-913.

[29]Wang LF, Zhang HY. A theoretical investigation on DPPH radical-scavenging mechanism of edaravone. *Bioorg Med Chem Lett* 2003; **13**(21): 3789-3792.

[30]Beyer FW, Fridovich I. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Anal Biochem* 1987; **161**: 559-566.

[31]Siddhuraju P. Manian S. The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. *Food Chem* 2007; **105**: 950-958.

[32]Wright JR Jr, Abraham C, Dickson BC, Yang H, Morrison CM. Streptozotocin dose response curve in tilapia, a glucose-responsive teleost fish. *Gen Comp Endocrinol* 1999; **114**: 431-440.

[33]Asok Kumar K, Uma Maheswari M, Sivashanmugam AT. Hypoglycemic effect of *Ficus microcarpa* leaves (Chinese Banyan) on alloxan-induced diabetic rats. *J Biol Sci* 2007; **7**: 321-326.

[34]Norfarizan-Hanoon NA, Asmah R, Rokiah MY, Faridah H. Antihyperglycemic, hypolipidemic and antioxidant enzymes effect of *Stobilanthes crispus* juice in normal and streptozotocin-induced diabetic male and female rats. *Int J Pharmacol* 2009; **5**: 200-207.

[35]Caspary WF, Rhein AM, Creutzfeldt W. Increase of intestinal brush border hydrolases in mucosa of streptozotocin-diabetic rats. *Diabetologia* 1972; **8**: 412-414.

[36]Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. *Clin Chem* 2000; **46**: 2050-2068.

[37]Kavalali G, Tuncel H, Göksel S, Hatemi HH. Hypoglycemic activity of *Urtica pilulifera* in streptozotocin-diabetic rats. *J Ethnopharmacol* 2003; **85**(1): 169-172.

[38]Salil G, Nevin KG, Rajamohan T. Arginine rich coconut kernel protein modulates diabetes in alloxan treated rats. *Chem Biol Interact* 2011; **189**: 107-111.

[39]da Silva PM, Zoppi CC, Filiputti E, Silveira LR, Quesada I, Boschero AC, et al. Preliminary report: leucine supplementation enhances glutamate dehydrogenase expression and restores glucose-induced insulin secretion in protein-malnourished rats. *Metabolism* 2010; **59**(6): 911-913.

[40]Doi M, Yamaoka I, Nakayama M, Sugahara K, Yoshizawa F. Hypoglycemic effect of isoleucine involves increased muscle glucose uptake and whole body glucose oxidation and decreased hepatic gluconeogenesis. *Am J Physiol Endocrinol Metab* 2007; **292**(6): 1683-1693.