Indian Journal of Traditional Knowledge
Vol 21(2), April 2022, pp 323-331

Bombax ceiba calyces regulate carbohydrate and lipid digesting enzyme's actions, display insulin sensitizing and antioxidant activities *in vitro*: A nutritional and phytochemicals examination

Komati Anusha[a,b], Ajay Anand[a,b,c], Katragadda Suresh Babu[a,b] & Ashok Kumar Tiwari*[,a,b]

[a]Centre for Natural Products & Traditional Knowledge, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India

[b]Academy of Scientific & Innovative Research (AcSIR), Ghaziabad 201 002, U P, India

[c]Carver College of Medicine, Department of Pathology, University of Iowa, Iowa City, IA 52242 (USA)

E-mail: tiwari@csiriict.in; tiwari@iict.res.in

Received 08 January 2021; revised 12 November 2021

Calyces of *Bombax ceiba* (*Śālmali*, *शाल्मली*) are used as vegetable by traditional Indian folks. However, scientific investigations for this vegetable are not available in literature. This research presents nutritional and phytochemicals composition in raw calyxes' powder and antihyperglycemic, antihyperlipidemic, insulin-sensitizing and, antioxidant properties in aqueous methanolic extract of calyx applying various *in vitro* methods. Calyx powder displayed rich source of macro- and micronutrients with energy value 322 Kcal. /100 g. Aqueous methanol extract of calyces exhibited potent antihyperglycemic activities by inhibiting pancreatic α-amylase and intestinal α-glucosidase enzymes, antihyperlipidemic by inhibiting pancreatic lipase and insulin sensitizing property by inhibiting PTP1β. It was found to be potent scavenger of chemical and physiological free radicals and, presented antioxidant activities by preventing H_2O_2 induced erythrocytes membrane lipid peroxidation and hemolysis. It also prevented H_2O_2 induced genomic DNA damage. The calyx powder presented 979 µg/100 g total antioxidant value. This report presents original analysis of nutritional and phytochemical composition along with biological activities in *B. ceiba* calyx.

Keywords: α-amylase, α-glucosidase, Antioxidant property, *Bombax ceiba* calyces, DNA damage, Lipase, Nutritional analysis, PTP1β inhibition

IPC Code: Int Cl.[22]: A61K 36/00, A61Q 19/08

Bombax ceiba L. (Syn. *Salmalia malabarica* Schott. & Endl.; *Bombax malabarica* DC, fam. Bombacaceae) is a fast growing deciduous tree. In Ayurvedic classics, it is known as *Śālmali* (Sanskrit). The root, stem bark, gum, leaves, flower, young fruits, seeds and, prickles of *B. ceiba* find multiple therapeutic mentions in various Ayurvedic texts[1]. This medicinal plant has been found useful in curbing number of age related morbidities and promote healthy ageing. Therefore, it has been envisioned as possible nutraceuticals for age management[2].

The glycation of proteins are natural but a slow process *in vivo* and is held accountable for ageing. It trounces functions of proteins and wanes elasticity of tissues like blood vessels, skin and tendons[3]. Hyperglycemia and oxidative stress is known to stimulate the process of proteins' glycation[4]. Buildup of advanced glycation end products (AGEs) results development of number of diabetic complications and accelerates ageing process[5]. Although the accumulation of AGEs is independent risk factor for cardiovascular disease development in ageing process, hyperlipidemia further aggravates the concerns[6]. These processes are shown to be slowed down by consumption of proper nutrition, healthful diet and physical activities[7].

The fleshy calyx of *B. ceiba* is utilized as vegetable in India by traditional folks[8]. It is rich in calcium, phosphorus and magnesium[9]. Interestingly, antiglycation activities and phytochemicals responsible for such activities have been identified recently in calyx of *B. ceiba*[10]. Nutrition is one of the most significant aspects of oxidative stress. Its features, diversity, consistency, ratio of different nutrients, dietary balance in proteins, carbohydrates, fats, macronutrients, micronutrients composition and, trace elements immensely help balance the physiological and biochemical processes governing healthy life[11]. These compositions in a whole dietary material facilitate

*Corresponding author

maintain the biochemical and physiological homeostasis in the body. Presence of anti-hyperglycemic, antihyperlipidemic, antioxidant activities together with macro- and micro nutrients in a dietary material therefore, makes it ideal food stuff for maintaining healthy body and preserves the biochemical and physiological homeostatic equilibrium.

Overt oxidative stress is the common soil ensuing metabolic disorders, especially in insulin-resistant hyperglycemic[12] and hyperlipidemic states[13]. Therefore, digestion of food materials and balanced utilization of metabolized food is prime aspect of health maintenance. Regulation of carbohydrate digesting enzyme, α-amylase and α-glucosidase, lipid digesting enzyme, pancreatic lipase and protein tyrosine phosphatase 1β (PTP1β) for insulin sensitization has been important aspects to control development of metabolic disorders. Presence of antioxidant properties in diet pays additional remuneration in devising holistic nutritional strategies to the problem. Presence of these biological activities in a dietary material thus, may promote healthy eating habits, selection of wholesome dietary material and ultimately maintaining good health.

In this research, we analyzed macro and micro-nutritional compositions, important therapeutic activities bearing phytochemicals in whole calyx of *B. ceiba* and assessed its potentials in regulating carbohydrate digesting enzyme, α-amylase for antihyperglycemic, lipid digesting enzyme pancreatic lipase for antihyperlipidemic and PTP 1β for insulin sensitization activities *in vitro*. Furthermore, the antioxidant activities were also, studied utilizing various *in vitro* test methods.

Materials and Methods

Folin-Ciocalteu reagent, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ethylenediam-inetetraacetic acid (EDTA), Nitro blue tetrazolium (NBT), riboflavin, sodium nitroprusside, Griess reagent, hydrogen peroxide (H_2O_2), thiobarbituric acid (TBA), 2-Deoxy-D-ribose, ascorbic acid, porcine pancreatic lipase type II, riboflavin, porcine pancreatic α-amylase, soluble potato starch, 3,5-dinitrosalicylic acid (DNS), rat intestinal acetone powder as a source of α-glucosidase, 4-nitroplenyl α-D-glucopyranoside, dithiothretol (DTT), β-mercaptoethanol, sodium orthovanadate, sucrose, acarbose, 4-nitrophenyl phosphate were purchased from Sigma-Aldrich chemicals (USA). Other chemicals of analytical grade were purchased from Indian manufacturers.

Plant collection and sample preparation

B. ceiba calyces were procured from northern part of India in the month of April 2017. Taxonomic identification was carried out by Dr. I. C. Barua, Department of Agronomy, Assam Agricultural University Jorhat (India). Voucher specimen with accession number 5297 is deposited in the departmental herbarium. Calyces were shed dried, fined powdered using a food-grade mixer grinder and stored in an air-tight container. The aqueous methanol extract (BCE) of powder was prepared by cold maceration process incubating the powder with water and methanol in equal volume for 72 h. Extract was dried using a rotary evaporator and stored at room temperature for further studies.

Nutritional and phytochemical analysis

B. ceiba calyx powder was used for nutritional and phytochemical analysis. The nutritional and phytochemical investigations of *B. ceiba* calyx (BCC) powder were carried out in Ministry of Food Processing Industries (MFPI)-Quality Control Laboratory of Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad. Detailed methods of nutritional and phytochemical analysis are described in supplementary file.

α-Amylase inhibition assay

Fourty µL of BCE (5 mg/mL, dissolved in DMSO) was incubated with 200 µL of porcine pancreatic α-amylase (4U/mL, prepared in Milli-Q water) for 5 min. The reaction was started by the addition of 400 µL soluble potato starch solution (0.5%w/v, prepared in 20 mM sodium phosphate buffer, *p*H 6.9). Exactly 3 min after reaction, 400 µL DNS color reagent was added. Tubes were placed in a hot-water bath (85-90°C) for 10 min to stop the reaction. A control was run sham in absence of test material. After cooling at room temperature, α-amylase activity was determined measuring absorbance of the mixture at 5 40 nm spectrophotometrically at BioTek synergy 4 multimode microplate reader (BioTek Instruments Inc, Winooski, VT, USA)[14]. Acarbose was taken as standard α-amylase inhibitor. The activity was calculated as follows: $(A_c-A_t)/100*A_c$, where A_c was the absorbance of control and A_t, the absorbance of test sample.

α-Glucosidase inhibition assay

20 µL of BCE (5 mg/mL, dissolved in DMSO) was incubated with 50 µL of rat intestinal α-glucosidase

enzyme (89.93 mM, prepared in 0.9% NaCl) in 100 mM Phosphate buffer (*p*H 6.8) for 10 min. after incubation period 50 µL of 4-nitroplenyl α-D-glucopyranoside solution was added. α-Glucosidase activity was determined measuring absorbance of the mixture at 405 nm spectrophotometrically[14]. Acarbose was taken as standard α-glucosidase inhibitor. The activity was calculated as follows: $(A_c-A_t)/100*A_c$, where A_c was the absorbance of control and A_t, the absorbance of test sample.

Pancreatic lipase inhibition

Pancreatic lipase inhibition was determined according to procedure described earlier[15]. Porcine pancreatic lipase type II dissolved in Milli-Q water was pre-incubated for 30 min with 50 µL BCE (5 mg/mL, dissolved in DMSO) and mixture was reacted with p-nitrophenyl laurate substrate prepared in sodium acetate buffer (20 mM, *p*H 5.0) for 2 h at 37°C. Orlistat was taken as standard lipase inhibitor. Enzyme activity was recorded by measuring release of 4-nitrophenyl spectrophometrically at 405nm as above.

PTP1β inhibition

PTP1β activity was determined as per previous method[16] with suitable modifications. Rat liver tissue was collected from sacrificed animal and homogenized in cold 0.1 M Phosphate Buffer Saline (PBS, *p*H 7.2) with the addition of cocktail protease inhibitor and centrifugation was performed for 30 min at 13000 rpm at 4ºC. Cytosolic supernatant was mixed with 10 µL of BCE (5 mg/mL, dissolved in DMSO) and incubated for 5 min. After incubation, 40 µL of substrate (*p*-nitrophenyl phosphate), and 130 µL buffer (25 mM Tris-HCl, pH 7.5 containing 2 mM β-mercaptoethanol, 1 mM EDTA and 1 mM Dithiothretol) were added. Reaction mixture was incubated at 37°C for 30 min. Sodium orthovanadate was run as standard inhibitor of enzyme. Released *p*-nitrophenol was recorded spectrophoto-metrically at 405 nm. Enzyme activity was determined as above.

Free radicals scavenging activities

Scavenging of $ABTS^{+}$ cation by BCE was recorded in phosphate buffer saline (6.8 mM, *p*H 8)[17]. Absorbance was measured at 734 nm spectrophotometrically. Similarly, the DPPH radical scavenging activities by the extract were determined in tris-HCl buffer (*p*H 7.4) by recording absorbance at 517 nm spectrophotometrically. A control was run sham without addition of extract. Ascorbic acid was used as standard. Radical scavenging by the *B. ceiba* extract was calculated applying formula $(A_c-A_t)/100*A_c$, where A_c was the absorbance of control and A_t, the absorbance of test sample.

Reduction of NBT

NBT reducing activity in extract was determined to verify reducing capacity[18]. Briefly, in a 96-well plate containing 100 µL phosphate buffer (50 mM, *p*H 10) equal quantity of NBT (1 mM, prepared in the same buffer) and 50 µL of BCE (5 mg/mL) were mixed. Mixture was incubated for 15 min. Reduction of NBT was measured spectrophotometrically at 560 nm. Percentage of NBT reduction by extract was calculated accordingly.

$FeCl_3$ reducing power assay

Reducing power of BCE was determined as per the previous method[18]. 100 µL of BCE (5 mg/mL) was mixed with 100 µL of 0.2 M phosphate buffer (*p*H 6.6) and 100 µL of potassium ferricyanide (1%). After 20 min incubation at 50°C, 10% trichloroacetic acid (TCA) was added to terminate the reaction. Mixture was centrifuged at 3,000 rpm for 10 min. Supernatant (100 µL) was transferred into a 96-well microplate and 100 µL of distilled water and 20 µL of 0.1% $FeCl_3$ were added and mixed well. Absorbance was measured spectrophotometrically at 700 nm and percentage of reducing power was calculated accordingly.

Scavenging of hydroxyl radical

20 µL of BCE (5 mg/mL in DMSO) was added to reaction mixture containing 100 µL of 2-Deoxy-D-ribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (10 mM), ascorbic acid (0.1 mM) and 400 µL KH_2PO_4-KOH buffer (20 mM, *p*H 7.4). Mixture was incubated for 1 h at 37°C. Reaction was terminated by addition of 1 mL TBA (1%) and heated at 95°C for 20 min. Thiobarbituric acid reactive substance formation was measured at 532 nm[19]. Percentage inhibition of hydroxyl radicals' formation was calculated accordingly.

Superoxide radical scavenging activity

Superoxides were generated in mixture containing 20 µL BCE (5 mg/mL in DMSO), 0.2 mL EDTA, 0.1 mL NBT and 0.05 mL riboflavin and 2.64 mL phosphate buffer (0.1 M, *p*H 7.2). DMSO was added in control instead of the extract. Absorbance was measured after 30 min incubation period at 560 nm spectrophotometrically[19]. Gallic acid was used as reference standard. Results were expressed as percentage inhibition of superoxide formation.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging assay was performed according to the previous method[19]. Reaction was initiated by adding 200 µL sodium nitroprusside, 50 µL Phosphate Buffer Saline (PBS, *p*H 7.4), 50 µL BCE (5 mg/mL prepared in DMSO) and incubated at 25°C for 30 min. 50 µL Griess reagent was added and again incubated for 30 min. DMSO was added in control instead of extract. Absorbance was read at 546 nm against reagent blank spectrophotometrically.

Erythrocytes hemolysis assay

Fresh blood was withdrawn in EDTA containing tubes from Wistar rats taking appropriate institutional ethical committee approval (IICT/IAEC/67/2018) and centrifuged at 1000 rpm for 20 min. Supernatant was discarded and pellet was washed with PBS (*p*H 7.4) to remove plasma, platelets and leucocytes etc. After washing erythrocytes were resuspended in PBS (*p*H 7.4) containing 2 mM sodium azide. 250 µL erythrocytes suspension was mixed with 250 µL BCE (5 mg/mL in DMSO) and 125 µL H_2O_2 (0.8M), incubated at 37°C for 60 min. After incubation mixtures were centrifuged at 3000 rpm for 3 min and absorbance of supernatant was recorded at 540 nm spectrophotometrically (release of hemoglobin)[20]. Inhibition of hemolysis was calculated comparing absorbance with control.

Erythrocytes membrane lipid peroxidation

Erythrocytes suspension (500 µL) was mixed with 500 µL BCE (5 mg/mL in DMSO) and 125 µL of H_2O_2. Reaction mixture was incubated for 1 h at 37°C. Following incubation, 1 mL of 20% of TCA was added to precipitate potentially interfering substances. Mixture was centrifuged at 3000 rpm for 3 min. Supernatant was mixed with 1 mL of 1% TBA and heated at 90°C for 50 min, cooled rapidly and centrifuged. Absorbance of the supernatant was measured at 532 nm spectrophotometrically[20]. Inhibition of lipid peroxidation by extract was calculated comparing absorbance values with control.

Genomic DNA damage

Protective effect of BCE on oxidative DNA damage was evaluated as per the previous method[21]. 2 µL calf-thymus DNA mixed with 5 µL 39 mM Tris buffer (*p*H 7.4) and 5 µL BCE (5 mg/mL in DMSO) and mixture was incubated at room temperature for 20 min. Reaction was initiated by adding 5 µL $FeCl_3$ (500 µM) and10 µL H_2O_2(0.8 M) and incubated for 10 min at 37°C. Reaction was stopped by adding 3 µL DNA loading dye. Finally, mixture was subjected to 0.8% agarose gel electrophoresis in TAE (40 mM Tris, 20 mM acetic acid and 0.5 M EDTA) buffer (*p*H 7.2). 3 µL of Ethidium bromide was added to agarose solution to stain DNA bands. Image was viewed under transilluminating UV light and photographed (Bio-Rad ChemiDocTM XRS with Image LabTM Software). Band intensity of DNA was measured by using ImageJ software.

Statistical analysis

One way ANOVA followed by Tukey's multiple comparison tests was applied to compare differences within the groups and unpaired t-test (two tailed) with Welch's correction was applied to compare differences between the groups. Criterion for statistical significance was set at $p<0.05$. Statistical analysis was performed by using GraphPad PRISM Version 5.01 (GraphPad Software Inc. California, USA).

Results and Discussion

Nutritional and phytochemical composition of *B. ceiba* calyx

Profiles of proximate compositions, vitamins, minerals, and phytochemicals in calyx powder are presented in Table 1. It is evident from the Table 1 that apart from macronutrients such as carbohydrates, protein, fat and sugars, it is rich source of minerals such as calcium, magnesium, iron, zinc and copper. Polyphenols, flavonoids and carotenoids are also present in ample amounts in the calyx. The total energy value of 100 g calyx powder was estimated to be 322 kilo calorie. Therefore, it presents a good source of healthy vegetable and snacks item.

The presence of abundant polyphenols in nutraceuticals and alternative therapies for diabetic and obese people has become an important consideration now. The potentials of dietary polyphenols in positively modifying impaired carbohydrate, lipid metabolism and, strengthening insulin sensitivity are among some of the important activities[22]. Polyphenols have been used to increase the function of fat tissue and play an important role in counteracting oxidative stress and stress-sensitive signal transduction pathways and, inflammatory processes[23]. Our study demonstrates that *B. ceiba* calyx powder is a rich source of polyphenols and flavonoids (Table 1). Vitamins and minerals play a significant role in regulating glucose metabolism and insulin signaling. Therefore, their deficiency in diet may lead development of metabolic disorders such as

Table 1 — Nutritional and phytochemical analysis of *B. ceiba* Calyx powder.

	Nutrients & Phytochemicals	Quantity
Proximate	Carbohydrate	71.3±0.01%
	Crude protein	6.84±0.05%
	Crude Fiber	18.7±0.08%
	Ash	9.26±0.04%
	Moisture	11.54±0.11%
	Total Fat	1.06±0.02%
Sugars	Total Sugars	13.23±0.15%
	Reducing Sugars	5.46±0.45%
	Acidity	0.13±0.55%
	Energy	322.10±0.58 (K.Cal/100 g)
Vitamins (mg/100 g)	Ascorbic Acid	6.06±0.08
	Pantothenic acid	5.60±0.02
	Riboflavin	0.51±0.02
Minerals (mg/100 g)	Iron	12.741±0.4
	Zinc	2.386±0.03
	Calcium	683.032±0.9
	Magnesium	641.628±0.98
	Sodium	22.023±0.01
	Copper	1.713±0.01
Phytochemicals (mg/100 g)	Total Polyphenols (GAE)	0.257±0.001
	Total Flavonoids (RE)	4.117±0.01
	Total carotenoids	0.449±0.05
	β-carotenoids	0.034 ±0.02
	Lycopene	0.00002 ±0.01
	Phytate	185.63±0.04
	Tannins	0.35 ±0.01
	Oxalate	3.58±0.04

Values represent mean±SD, n=3. All the experiments were repeated at least three times. GAE; gallic acid equivalent, RE; rutin equivalent.

diabetes and obesity[24]. Paucity of these important micronutrients and minerals has been reported compromised in diabetic and obese individuals[25]. Our analysis finds that *B. ceiba* calyx is rich with carbohydrate, protein, fat, fibre and potential source of minerals viz., iron, zinc, calcium, magnesium, sodium, copper and vitamin like ascorbic acid, pantothenic acid and riboflavin (Table 1).

Carotenoids are finding importance in diet due to their multiple health benefits[26]. *B. ceiba* calyx was found to be a good source of carotenoids including β-carotene and Lycopene in our study (Table 1). The polyphenols, flavonoids and carotenoids are natural sources of antioxidant molecules. Their presence in a diet offers health benefits in alleviating oxidative stress of multiple origins[26]. The total antioxidant activity in *B. ceiba* calyx powder in our analysis was found at 978.67 µg/100 g.

Pancreatic α- Amylase inhibition

The α-amylase is an enzyme that begins the process of carbohydrate digestion. The inhibition of pancreatic α-amylase is one of the therapeutic targets for delaying oligosaccharide digestion to absorbable monosaccharides in the intestinal brush border, resulting in reduced postprandial hyperglycemia[27]. Acarbose is a pseudosaccharide which inhibits carbohydrate digestion by slowing down the activity of polysaccharides digestion[28]. However, its undesirable effects have limited patients' likeness to use it. The realization that phytochemicals present in our dietary materials also possess similar activities without any untoward effects, spurred their identification and promotion. The α-amylase inhibitory activity by acarbose and BCE is presented in Table 2. BCE could inhibit activity of α-amylase however its potency was three times less than acarbose ($p<0.0006$). BCE is rich source of polyphenols and flavonoids (Table 1). These phytochemicals have been found to manipulate the activity of carbohydrate digesting enzymes[29] and may provide reasonable explanation for the activity observed in this study.

Intestinal α- glucosidase inhibition

α-Glucosidase enzyme is a key enzyme that catalyzes the digestion of disaccharides. Inhibition of α-glucosidase in the intestine delays digestion of disaccharides and hence slows down the postprandial increase in blood glucose[30]. It was observed that BCE inhibited digestion of substrate by 53.05±1.99% (Table 2). This action might be due to the presence of polyphenolic compounds or flavonoids (Table 1). Acarbose, the standard α-glucosidase inhibitor, reduced enzyme activity by 64.058±2.98% (Table 2). These results showed that BCE is a good choice for slowing down the digestion of carbohydrate.

Pancreatic lipase inhibition

Pancreatic lipase decreases the absorption of dietary fat due to the inhibition of triglyceride hydrolysis. Orlistat is a clinically approved drug to manage postprandial hyperlipidemia however, undesirable gastrointestinal disturbances limits its use by the individuals[31]. Interestingly, presence of pancreatic lipase inhibitors in dietary materials has shown promise in reducing postprandial hyperlipidemic excursions[32]. Although pancreatic lipase inhibition by BCE was observed to be in the close proximity of standard drug

Table 2 — Biological activities displayed by BCE

Parameters	% Activity by BCE[!]	% Activity (standard[!])	*p values
Pancreatic α-amylase inhibition	19.89±0.75	69.09±1.88 (Acarbose)	0.0006
Intestinal α-glucosidase inhibition	53.05±1.99	64.08±2.98 (Acarbose)	0.0026
Pancreatic lipase inhibition	14.59±0.219	22.36±0.15 (Orlistat)	0.0132
PTP 1β inhibition	29.738±0.49	93.99±4.33 (Sodium orthovanadate)	0.0042
$ABTS^{\bullet+}$ scavenging	99.0213±0.24	99.05±0.05 (Ascorbic acid)	ns
DPPH scavenging	82.71±1.35 [a]	92.38±0.24 (Ascorbic acid)	0.0067
$FeCl_3$ reduction	29.67±0.35 [a,b]	99.51±0.03 (Ascorbic acid)	0.0006
NBT reduction	77.05±2.05[b]	99.7±0.1 (Ascorbic acid)	0.0027
Hydroxyl radical scavenging	76±0.677[c,d]	85.1±0.57 (Gallic acid)	0.0114
Superoxide radical scavenging	58.80±1.60[c,e]	75.23±0.40 (Gallic acid)	0.0037
Nitric oxide	40.86±0.92[d,e]	41.59±1.93 (Gallic acid)	ns
Erythrocyte hemolysis	25.49±1.79[f]	35.22±0.350 (Ascorbic acid)	0.0115
Lipid peroxidation	34.59±1.42[f]	62.79±2.86 (Ascorbic acid)	0.0001

Values represent mean±SD, n=3. [!]Concentration of BCE and respective standards for particular activity was same. *Comparisons with BCE & respective standards. Differences between identical notations were observed as followed: [a]$p<0.0209$, [b]$p<0.006$, [c]$p<0.0034$, [d]$p<0.0001$, [e]$p<0.0005$, [f]$p<0.0062$. Unpaired t- test (two tailed) with Welch's correction was applied to compare between groups. ns=not significant.

orlistat (Table 2) in our analysis, statistically the difference was significant ($p<0.0132$). Therefore, the antihyperlipidemic activity present in BCE may help reduce diet induced hyperglycemic excursions.

PTP 1β inhibition

PTP 1β is a major negative regulator of insulin signaling pathway. It de-phosphorylates activation factors linking insulin receptors and insulin receptor signaling. Therefore, it is a central link in insulin signaling pathway. Interestingly in PTP 1β deficient mice, the insulin sensitivity was observed improved[33]. Therefore, PTP 1β inhibitors present prospective drug target to mitigate fundamentals of insulin resistance and thereby improve insulin sensitivity. Vegetables have been shown to present various degrees of PTP 1β inhibitory potentials corresponding to the presence of polyphenols in vegetable's juice[34]. In present study, BCE also could show close to 30% inhibition of PTP 1β (Table 2). Based on this observation, it can be envisaged that utilization of *B. ceiba* calyx in diet may improve insulin sensitivity in compromised cases.

Free radicals scavenging activities

Amphiphilic nature of $ABTS^{\bullet+}$ cation is used to identify hydrophilic and hydrophobic antioxidants in dietary materials[35], whereas, $DPPH^{\bullet}$ radical can determine the reducing power of an antioxidant[36]. These simple chemical assays provide preliminary information regarding radical scavenging and reducing powers of potential antioxidant candidates. BCE could scavenge these radicals potently in our screening (Table 2). The $ABTS^{\bullet+}$ cation scavenging activity by BCE was found to be same as that of the ascorbic acid (Table 2).When reducing power was tested on $FeCl_3$ reducing assay, it was more than two times less potent ($p<0.0209$) in displaying reducing power than that observed for DPPH radical reduction (Table 2). On the other hand reducing potential of BCE for NBT was observed twice better ($p<0.006$) than that for the $FeCl_3$ (Table 2). Such discrepancies arise in estimating antioxidant activities in a sample due to presence of various types of reductones (antioxidants) responsible for disruption of the free radicals chain reaction by transferring hydrogen atoms[37].

In biological system, hydroxyl radicals interact with fats, polypeptides, proteins and DNA, notably thiamine and guanosine[38]. Superoxide radical is recognized as a preliminary radical and plays important role in generation of other ROS[39]. The signaling molecule nitric oxide in normal physiological conditions acts as antiinflammatory molecule however it's over production under abnormal conditions such as oxidative stress changes its role as pro-inflammatory molecule[40].

The hydroxyl, superoxide and nitric oxide radicals scavenging potentials of BCE is presented in Table 2. BCE scavenged hydroxyl radicals more potently than superoxide ($p<0.0034$) and nitric oxide ($p<0.0001$). Similarly, superoxide radicals scavenging activity by BCE was more potent ($p<0.0005$) than observed for nitric oxide (Table 2). The nitric oxide radical

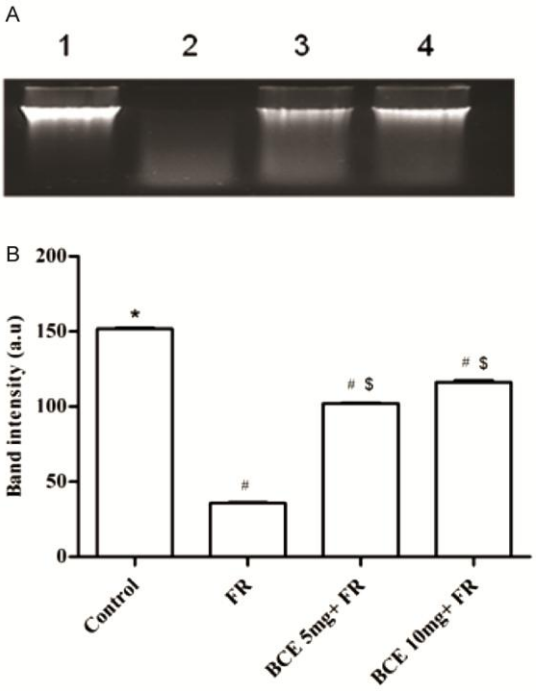

Fig. 1 — Inhibition of H_2O_2 induced DNA damage by BCE. (a) Lane-1, Control DNA; lane-2, Fenton's Reagent (FR); lane-3- BCE 5mg+FR; lane-4- BCE 10mg+FR, (b) Comparisons of band intensity of different lanes. One way ANOVA followed by Tukey's multiple comparison tests was applied to compare differences within the bars. Degree of statistical significance was set at $p<0.05$. $^{*}p<0.05$ when compared with band intensities of lanes-2, -3 & -4. $^{\#}p<0.05$ (band intensity of lane-2 vs. lane-3 and lane-4) and $\$p<0.05$ (band intensity of lane-3 vs. lane-4).

scavenging activity by BCE was in the close proximity of standard molecule gallic acid (Table 2). These results demonstrate that BCE possesses the potentials of scavenging physiologically damaging free radicals.

H_2O_2 induced hemolysis and lipid peroxidation

Free radicals development have been observed significantly elevated in hyperglycemic as well as hyperlipidemic individuals suggesting their contribution in inducing oxidative stress[41]. High concentrations of polyunsaturated fatty acids in erythrocytes membrane make them highly susceptible to the attack of free radicals[42]. Oxidative stress and free radicals attack on erythrocytes causes membrane's lipid peroxidation. The resultant membrane disruption leads hemoglobin's leakage called hemolysis. BCE could potently prevent both the H_2O_2 induced lipid peroxidation as well as hemolysis of erythrocytes (Table 2). The H_2O_2 induced lipid perxidation in erythrocytes membrane was potently ($p<0.0062$) inhibited by BCE than H_2O_2 induced erythrocytes hemolysis.

Protective effect on oxidative DNA damage

The exposure of calf-thymus DNA to Fenton's reagent results in strand breaks. Hydroxyl radicals react with nitrogenous bases of DNA generating base radicals and sugar radicals. The base radicals, in turn, react with the sugar moiety, which causes the rupture of the sugar phosphate backbone of nucleic acid, resulting in the rupture of the string[43]. The observations presented in Figure 1 shows that the addition of BCE along with Fenton's reagent provided significant degree ($p<0.05$) of protection to genomic DNA (Fig. 1b) and significantly ($p<0.05$) preserves its integrity in a concentration dependent manner (Fig. 1b). Genoprotective activity of BCE may be attributed to the presence of free radicals scavenging antioxidant phytoconstituents like flavonoids, polyphenols and carotenoids etc in BCE (Table 1).

Conclusion

Our study finds for the first time that *B. ceiba* calyx is rich with natural source of macro- and micronutrients, vitamins, minerals and phytochemicals like flavonoids, polyphenols and carotenoids. It possesses free radicals scavenging and displayed antioxidative activities in preventing free radicals-induced erythrocytes lipid peroxidation and hemolysis. Furthermore, it also prevented free radicals induced genomic DNA damage. Simultaneously, it also displayed its *in vitro* potentials as antihyperglycemic by inhibiting α-amylase, antihyperlipidemic due to pancreatic lipase inhibition and insulin sensitizing activities by inhibiting activity of PTP 1β. These observations provide promising opportunities for *B. ceiba* calyx to be used as vegetable and functional food for people suffering from metabolic disorders. To validate these findings animal studies and clinical observations are further required.

Acknowledgement

Authors thank Director, CSIR-IICT for support (IICT/Pubs./2020/373) and providing research facilities. KA acknowledges Council of Scientific and Industrial Research, New Delhi for providing SRF and AA to University Grants commission, New Delhi for awarding SRF.

Conflict of Interest

All the authors declare that they have no conflict of interest financial or otherwise.

Authors' Contributions

KA & AA contributed in experimental analysis, methodology, data generation, interpretation and

preparation of manuscript drafts. KSB & AKT were involved in concept development, experiment design, data analysis, data interpretation, manuscript writing, editing, finalization and funding arrangements.

References

1 Meena V, Shalmali (*Bombax ceiba*): Versatility in its therapeutics, *Int J Green Pharm*, 11 (03) (2017) S401-S406.

2 Ndidi C Ngwuluka, Are *Bombax buonopozense* and *Bombax malabaricum* possible nutraceuticals for age management? *Prev Med*, 54 Suppl (2012) S64-70.

3 Semba R D, Nicklett E J, Ferrucci L, Does accumulation of advanced glycation end products contribute to the aging phenotype?, *J Gerontol A Biol Sci Med Sci*, 65 (2010) 963-75.

4 Ahmed N & Thornalley P J, Quantitative screening of protein biomarkers of early glycation, advanced glycation, oxidation and nitrosation in cellular and extracellular proteins by tandem mass spectrometry multiple reaction monitoring, *Biochem Soc Trans*, 31 (2003) 1417–22. doi: 10.1042/bst0311417.

5 Suji G & Sivakami S, Glucose, glycation and aging, *Biogerontology*, 5 (2004) 365-73. doi: 10.1007/s10522-004-3189-0.

6 Fishman S L, Sonmez H, Basman C, Singh V & Poretsky L, The role of advanced glycation end-products in the development of coronary artery disease in patients with and without diabetes mellitus: a review, *Mol Med*, 24 (59) (2018) 1-12.

7 Chan-Sik K, Sok P & Junghyun K, The role of glycation in the pathogenesis of aging and its prevention through herbal products and physical exercise, *J Exerc Nutr Biochem*, 21 (3) (2017) 55-61.

8 Jain V & Verma S K, *Pharmacology of Bombax ceiba Linn*, Springer Science & Business Media, (2012) p. 11-23. DOI: 10.1007/978-3-642-27904-1_2.

9 Bhatnagar M S, Chemical composition of calyx of Salmalia malabarica, *Sci Cult*, 1965, 31, 189.

10 Komati A, Anand A & Shaik H, *Bombax ceiba* (Linn.) calyxes ameliorate methylglyoxal-induced oxidative stress via modulation of RAGE expression: identification of active phytometabolites by GC-MS analysis, *Food Funct*, 11 (6) (2020) 5486-5497.

11 Chung H C, Sung S H & Kim J S, Lack of cytochrome P450 2E1 (CYP2E1) induction in the rat liver by starvation without coprophagy, *Drug Metab Dispos*, 29 (3) (2001) 213-216.

12 Ormazabal V, Nair S & Elfeky O, Association between insulin resistance and the development of cardiovascular disease, *Cardiovasc Diabetol*, 17, 122 (2018).

13 Yun J W, Possible anti-obesity therapeutics from nature–A review, *Phytochem*, 71 (14-15) (2010) 1625-1641.

14 Srujana M S, Thota R S P & Anand A, *Raphanus sativus* (Linn.) fresh juice priming moderates sucrose-induced postprandial glycemia as well as a postprandial glycemic excursion in rats, *Indian J Tradit Know*, 18 (2) (2019) 339-345.

15 McDougall G J, Kulkarni N N, & Stewart D, Berry polyphenols inhibit pancreatic lipase activity in vitro, *Food Chem*, 115 (1) (2009) 193-199.

16 Klomann S D, Mueller A S & Pallauf J, Antidiabetic effects of bitter gourd extracts in insulin-resistant db/db mice, *Br J Nutr*, 104 (11) (2010) 1613-1620.

17 Reddy S V, Tiwari A K & Kumar U S, Free radical scavenging, enzyme inhibitory constituents from antidiabetic Ayurvedic medicinal plant *Hydnocarpus wightiana* Blume, *Phytother Res*, 19 (4) (2005) 277-281.

18 Tiwari A K, Manasa K & Kumar D A, Raw horse gram seeds possess more in vitro antihyperglycaemic activities and antioxidant properties than their sprouts, *Nutrafoods*, 12 (2) (2013) 47-54.

19 Haida Z & Hakiman M A, comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities, *Food Sci Nutr*, 7 (5) (2019) 1555-1563.

20 Lalitha S & Selvam R, Prevention of H_2O_2- induced red blood cell lipid peroxidation and hemolysis by aqueous extracted turmeric, *Asia Pac J Clin Nutr*, 8 (2) (1999) 113-114.

21 Chang M, Bellaoui M & Boone C, A genome-wide screen for methyl methanesulfonate-sensitive mutants reveals genes required for S phase progression in the presence of DNA damage, *Proc Natl Acad Sci*, 99 (26) (2002) 16934-16939.

22 Mihaylova D, Popova A & Alexieva I, Polyphenols as Suitable Control for Obesity and Diabetes, *Open Biotech J*, 12 (2018) 219-228 DOI: 10.2174/1874070701812010219.

23 Bahadoran Z, Mirmiran P & Azizi F, Dietary polyphenols as potential nutraceuticals in management of diabetes: a review, *Diabetes Metab Syndr*, 12 (1) (2013) 43.

24 Via M, The malnutrition of obesity: micronutrient deficiencies that promote diabetes, *Int Sch Res Notices*, 2012, Article ID 103472, doi:10.5402/2012/103472

25 Walker A F, Potential micronutrient deficiency lacks recognition in diabetes, *Br J Gen Pract*, 57 (534) (2007) 3-4.

26 Krinsky N I & Johnson E J, Carotenoid actions and their relation to health and disease, *Mol Aspects Med*, 26 (6) (2005) 459-516. doi: 10.1016/j.mam.2005.10.001.

27 Slanc P, Doljak B & Kreft S, Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition, *Phytother Res*, 23 (2009) 874-877.

28 Balfour J A & McTavish D, Acarbose: An update of its pharmacology and therapeutic use in diabetes mellitus, *Drugs*, 46 (6) (1993) 1025-1054.

29 Deepthi S, Anusha K & Anand A, Micronutrients and phytochemicals content in various rice (*Oryza sativa* Linn.) samples control carbohydrate digestion variedly and present differential antioxidant activities: an in vitro appraisal, *Indian J Tradit Know*, 19 (4) (2020) 821-831.

30 Kumar S, Narwal S, Kumar V & Prakash O, α-glucosidase inhibitors from plants: A natural approach to treat diabetes, *Pharmacogn Rev*, 5 (2011) 19-29.

31 Heck A M, Yanovski J A & Calis K A, Orlistat, a new lipase inhibitor for the management of obesity, *Pharmacother*, 20 (3) (2000) 270-279.

32 Klaman L D, Boss O & Peroni O D, Increased energy expenditure, decreased adiposity and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice, *Mol Cell Biol*, 20 (15) (2000) 5479-5489.

33 Huang D, Ou B & Prior R L, The chemistry behind antioxidant capacity assays, *J Agric Food Chem*, 53 (6) (2005) 1841-1856.

34 Tiwari A K, Kumar D A & Sweeya P S R, Protein-Tyrosine Phosphatase 1B inhibitory activity potential in vegetables' juice, *Pharmacologia*, 4 (4) (2013) 311-319. DOI: 10.5567/pharmacologia.2013.311.319

35 Prior R L, Wu X & Schaich K, Standardized methods for the determination of antioxidant capacity and phenolics in foods

and dietary supplements, *J Agric Food Chem*, 53 (10) (2005) 4290-4302.

36 Meir S, Kanner J, Akiri B & Philosoph-Hadas S, Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves, *J Agric Food Chem*, 43 (7) (1995) 1813-1819.

37 Ashok B T & Ali R, The aging paradox: free radical theory of aging, *Exp Gerontol*, 34 (3) (1999) 293-303.

38 Stief T W, The physiology and pharmacology of singlet oxygen, *Med Hypotheses*, 60 (4) (2003) 567-572.

39 Miller M J, Sadowska-Krowicka H A & Chotinaruemol S O, Amelioration of chronic ileitis by nitric oxide synthase inhibition, *J Pharmacol Exp Ther*, 264 (1) (1993) 11-16.

40 oxide in inflammatory diseases, *Inflammopharmacology*, 15 (6) (2000) 252-259. doi: 10.1007/s10787-007-0013-x.

41 Maritim A C, Sanders A & Watkins Iii J B, Diabetes, oxidative stress and antioxidants: A review, *J Biochem Mol Toxicol*, 17 (1) (2003) 24-38.

42 Abirami A, Nagarani G & Siddhuraju P, In vitro antioxidant, anti-diabetic, cholinesterase and tyrosinase inhibitory potential of fresh juice from *Citrus hystrix* and *C. maxima* fruits, *Food Sci Hum Well*, 3 (1) (2014) 16-25.

43 Lloyd D R & Phillips D H, Oxidative DNA damage mediated by copper (II), iron (II) and nickel (II) Fenton reactions: evidence for site-specific mechanisms in the formation of double-strand breaks, 8-hydroxydeoxyguan-osine and putative intra strand cross-links, *Mutat Res Funda Mol Mec Mutagen*, 424 (1-2) (1999) 23-36.