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Health related functional characteristics and antioxidant potential of mucilage (dietary fiber) from *Zizyphus mauritiana* fruits

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Abstract

The composition of *Zizyphus mauritiana* mucilage (ZMM) and several properties related to its nutritional quality were investigated. The ZMM with good water holding capacity (25.21 g/g), swelling capacity (19.34 mL/g), glucose dialysis retardation index and *in vitro* amylolysis kinetics indicate that it may have the capability in controlling the diabetes. Structural characteristics of ZMM were analyzed using the Fourier transform infrared (FT-IR) spectroscopy. In addition, the ZMM has the strong antioxidant potency against ABTS (16,587.32 mmol trolox equiv./g), DPPH (5.27 g mucilage/g DPPH), hydroxyl (76.13%) and superoxide (85.12%) radicals due to the presence of polyphenols (25.54 mg GAE/g mucilage). Besides, the ZMM also inhibited the acetylcholine esterase (86.89%), tyrosinase (47.01%), α -amylase (70.13%) and α -glucosidase (87.14%) enzymes. These properties make the crude mucilaginous fraction from *Z. mauritiana* fruit an excellent candidate of potential nutraceutical and functional food.

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Keywords: *Zizyphus mauritiana* mucilage; Functional characteristics; Diabetes; Fourier transform infrared (FT-IR); Functional food

1. Introduction

High fiber foods start to gain much attention in the industrial world, due to its importance in human health. Finding new and cheap sources of dietary fiber is extensively needed in developing countries to maintain populational health. The dietary fiber (DF) exists as the soluble or insoluble form; most of the plant foods have the combination of both. The DF endeavors a beneficial role in human health, mainly due to its following attributed qualities. Food rich in DF is unchanged/indigested in the small intestine and it entraps/absorbs the organic molecules (e.g. glucose), which play a potential role in controlling the diabetes. After being absorbed in the large intestine, it is fermented and provides carbohydrates to the colonic bacteria and

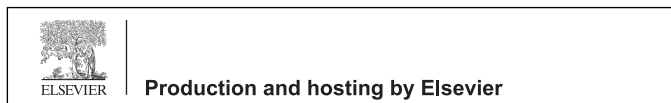
produces short fatty acid that helps to maintain the colon health [1]. Besides, plant-derived DF are also enriched in phytoconstituents which can be considered as dietary antioxidants. Thus, the consumption of diet enriched with DF helps to maintain or reduce the incidence of common disorders such as diabetes, obesity and colon cancer. Most fruits, vegetables and whole grains are good sources of DF. Fruits have relatively more soluble DF (mucilage), i.e. a higher ratio soluble/low insoluble fiber [2]. Mucilage is a high molecular weight polyuronides consisting of sugar and uronic acid units. It is partially soluble in water and can form highly viscous solution. It exhibits hampering effect on the diffusion of glucose, and helps to postpone the absorption and digestion of carbohydrates which results in lowered postprandial blood glucose [3].

Zizyphus mauritiana (Indian jujuba) of Rhamanceae family is an indigenous fruit tree of India and grows under varying conditions of climate all over India even at elevations up to 1000 m. *Z. mauritiana* fruits are edible and contain significant levels of important nutrients, including vitamins, minerals, fiber and protein. Interestingly, the content of nutrients in *Z. mauritiana* was found to be higher than that of some other fruits like mango, guava, orange and strawberry [4]. In addition, phytonutrient-enriched *Z. mauritiana* fruits also exhibited antioxidant activity [5]. Despite above mentioned facts, the cheap but nutritive *Z. mauritiana* still remains underutilized in Indian diet mainly because the consumers have no scientific information about

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its health promoting effects. The isolated polysaccharides of *Zizyphus jujuba* (Chinese jujuba) has been characterized, showing antioxidant, hepatoprotective and anti-inflammatory activity [6–8]. Most of the information in literatures has been restricted to Chinese jujuba while Indian jujuba requires further research.

Keeping this in mind, the present study isolated the crude mucilage from *Z. mauritiana* fruit (ZMM) and evaluated its antioxidant activity and functional properties in association with *in vitro* anti-diabetic effects. Detailed information about the structural and functional characteristics helps us in better understanding its physiological effects and health benefits.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals used in this study were of analytical grade. 2',2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), butylated hydroxyanisole (BHA), 2,2-diphenyl-1-picryl hydrazyl (DPPH), α -amylase, α -glucosidase, tyrosinase, acetylcholinesterase, *p*-nitrophenyl- α -D-glucopyranoside, L-DOPA, acetylthiocholine iodide and *p*-nitrophenyl- β -D-glucuronide were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were obtained from HiMedia Laboratories (Mumbai, India). Water was treated by arium 67316 reverse osmosis (Sartorius Stedim Biotech GmbH, Germany). All spectrophotometric measurements were done using UV-Vis 100 (Cyberlab, USA).

2.2. Mucilage extraction

The ripen fruits of *Z. mauritiana* were purchased from local markets of Coimbatore, Tamilnadu in January 2010. The fruits were thoroughly washed with water to remove dirt and debris. The fresh peel and pulp were separated from seed and mixed with water at a ratio of 1:10. The mixtures were blended using the laboratory blender (Remi Anupam Mixie Ltd, Mumbai, India) and was centrifuged with $3000 \times g$ for 30 min. The supernatant was collected and the absolute ethanol was added to the supernatant at the ratio 1:2 to precipitate the mucilage. Then the crude mucilage obtained were collected, dried in the oven at 40°C and ground to fine powder using laboratory blender followed by ball mill MM400 (Retsch, Germany). The powdered crude mucilage was stored in room temperature for further analysis.

2.3. Functional properties

2.3.1. Swelling capacity

0.5 g mucilage powder was precisely weighed into a calibrated cylinder, the volume of solid was recorded (V_1) and 10 mL distilled water was added. After mixing, the mixture was left to stand at room temperature for 18 h. The bed volume was recorded (V_2). Swelling capacity (SC) was expressed as milliliter per gram of mucilage (mL/g) [9] and calculated as follows:

$$\text{SC (mL/g)} = \frac{V_2 - V_1}{0.5}$$

2.3.2. Water and oil holding capacity

Ten milliliters distilled water or refined sunflower oil was added to 0.5 g of three series (triplicate) of mucilage powder in centrifuge tubes. The mixture was stirred carefully and left to stand at room temperature for 60 min. After centrifugation at $3000 \times g$ for 15 min, the supernatant was carefully discarded, and the residue was weighed (m). Water holding capacity (WHC) and oil holding capacity (OHC) were expressed as the amount of water or oil retained per gram of mucilage powder (g/g) [9]. The WHC and OHC were calculated as follows:

$$\text{WHC/OHC (g/g)} = \frac{m - 0.5}{0.5}$$

2.3.3. Emulsifying properties

The emulsifying activity and stability were determined in triplicates by the methods of Neto et al. [10]. Five milliliters of mucilage dispersion in distilled water (10 mg/mL) was homogenized (1 min) with 5 mL refined sunflower oil. The emulsions were centrifuged ($1100 \times g$, 5 min) and the height of the emulsified layer as well as the height of total contents in the tube was determined. The emulsifying activity was calculated as follows:

$$\text{Emulsifying activity (\%)} = \frac{\text{Height of the emulsified layer}}{\text{Height of the total content}} \times 100$$

Emulsion stability (ES) was determined by heating the emulsion (80°C , 30 min) before centrifuging ($1100 \times g$, 5 min) and calculated as follows:

$$\text{ES (\%)} = \frac{\text{Height of the emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \times 100$$

The effect of concentration on emulsifying activity and stability of mucilage was studied by preparing 6% (m/V) solution before conducting experiments described above.

2.3.4. Determination of glucose adsorption capacity

Glucose adsorption capacity was determined according to Ou et al. [3] with slight modifications. A sample of 1 g was mixed with 100 mL glucose solution (concentrations from 10 to 200 mmol/L) and incubated at 37°C for 6 h. When the adsorption reached equilibrium, the sample was centrifuged at $4000 \times g$ for 20 min. The glucose content in the supernatant was then determined using glucose assay kit (Sigma–Aldrich, St. Louis, MO, USA). The adsorbed glucose was calculated as the amount of glucose retained by the sample (mmol/g of fiber).

2.3.5. Determination of glucose dialysis retardation index (GDRI)

On the basis of the methods of Ou et al. [3] with slight modifications, a mucilage sample (0.5 g), xanthan gum and guar gum (0.5 g) was mixed with 25 mL glucose solution (50 mmol/L), and the mixture solution was dialyzed against 100 mL distilled water at 37°C using a dialysis membrane with a cut off molecular weight of 12,000 Da. After 30, 60, 90 and 120 min, the

glucose content in the dialysate was measured using the glucose assay kit to determine the GDRI as a function of time. A control test was carried out without the addition of fiber. The GDRI was calculated with the following equation:

$$\text{GDRI} = 100 - \left[\frac{\text{glucose content in the sample with the addition of fiber}}{\text{glucose content of the control}} \times 100 \right]$$

2.3.6. Effect of the samples on amylolysis kinetics *in vitro*

Forty grams of potato starch were added to 900 mL of 0.05 mol/L phosphate buffer (pH 6.5). The solution, after stirring at 65 °C for 30 min, was made up to a final volume of 1000 mL to give a 4% (m/V) starch solution. A starch- α -amylase DF system comprising the above starch solution (25 mL), α -amylase (0.4%), and test samples (1%) were dialyzed in a dialysis bag against 200 mL distilled water at 37 °C (pH 7.0) in a shaker water bath. The glucose content in the dialysate was determined at 20, 30, 60, 120 and 180 min, respectively. A control test was carried out without the addition of the sample [3].

2.3.7. Scanning electron microscopy (SEM)

A powder samples of ZMM was loaded on a conducting double-sided carbon tape, sputter coated with gold palladium and scanned using ICON analytical, FEI QUANTA 200.

2.3.8. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum was used to analyze the chemical structure of the polysaccharide. Fourier transform infrared data was obtained using the Bruker Tensor 27 FTIR spectrophotometer. The spectrum was recorded in the attenuated total reflectance (ATR) mode in the range of 600–4000 cm^{-1} .

2.4. Phytochemical analysis and antioxidant activity

2.4.1. Total phenolic content

The total phenolics content in the crude mucilage was determined according to the method described by Siddhuraju and Becker [11]. Known aliquots of the sample solution were taken in a test tube and made up to a volume of 1 mL with distilled water. It was mixed sequentially with 0.5 mL Folin–Ciocalteu (1:1 diluted with water) reagent and 2.5 mL sodium carbonate solution (20%, m/V). The mixture was vortexed and incubated in the dark for 40 min and the absorbance was recorded at 725 nm against a reagent blank. The analysis was performed in triplicates, and the results were expressed as gallic equivalents.

2.4.2. Free radical scavenging activity on DPPH

The radical scavenging activity of extracts was measured using DPPH radical by the method of Sanchez-Moreno et al. [12] with slight modifications. The sample solution of 0.1 mL was mixed with 3.9 mL of DPPH \cdot (0.025 g/L) and incubated in the dark for 30 min. Absorbance was measured at 515 nm and the result was expressed as g extract/g DPPH.

2.4.3. Free radical scavenging activity on ABTS \bullet^+

The ABTS cationic radical (ABTS \bullet^+) decolorization assay was done by the method of Re et al. [13]. ABTS \bullet^+ was generated by adding 2.45 mmol/L potassium persulphate (final concentration) to 7 mmol/L ABTS and incubated in the dark at room temperature for 12–16 h. The stock solution of ABTS \bullet^+ was diluted with ethanol to give an absorbance of 0.70 ± 0.02 at 734 nm (working solution). 10 μL sample was mixed with 1.0 mL ABTS \bullet^+ working solution and incubated at 30 °C for 30 min and the absorbance was measured at 734 nm. The results were expressed as mmol trolox equiv./g extract.

2.4.4. Hydroxyl radical scavenging activity

Hydroxyl radical ($\bullet\text{OH}$) scavenging activity of extracts was measured using the ascorbic acid-iron-EDTA assay [14]. 200 μg sample was mixed with 1 mL iron-EDTA solution (0.13% ferrous ammonium sulphate in 0.26% EDTA), 0.5 mL of 0.018% EDTA and 1 mL of 0.85% dimethyl sulfoxide (DMSO) solution (in 0.1 mol/L phosphate buffered saline, pH 7.4). The reaction was initiated by the addition of 0.5 mL of 0.22% ascorbic acid and incubated at 80–90 °C in water bath for 15 min. After incubation, the reaction was terminated by the addition of 1 mL ice cold trichloroacetic acid (TCA; 17.5 m/V). 3 mL Nash's reagent (containing 75 g ammonium acetate, 3 mL glacial acetic acid and 2 mL acetyl acetone per liter) was added to the above mixture. And the mixture was allowed to stand at room temperature for 15 min for color development. Absorbance was recorded at 412 nm. The hydroxyl radical scavenging activity (HRSA) was calculated using the following formula:

$$\text{HRSA} (\%) = \frac{A_c - A_s}{A_c} \times 100$$

2.4.5. Superoxide anion radical scavenging activity

The superoxide anion radical ($\text{O}_2^{\bullet-}$) scavenging capacity of extracts was determined by the method of Martinez et al. [15]. Each 3 mL reaction mixture consisting of 50 mmol/L phosphate buffer (pH 7.8), 13 mmol/L methionine, 2 $\mu\text{mol/L}$ riboflavin, 100 $\mu\text{mol/L}$ EDTA, 75 $\mu\text{mol/L}$ nitroblue tetrazolium chloride (NBT) and 1 mL sample solution (150 $\mu\text{g/mL}$) is kept for 10 min under illumination with a 20 W fluorescent lamp. The production of blue formazan was monitored and recorded at 560 nm. The degree of superoxide radical scavenging activity was calculated as follows:

$$\text{Scavenging activity} (\%) = \frac{A_c - A_s}{A_c} \times 100$$

2.5. Analysis of various enzyme inhibitory activities *in vitro*

2.5.1. α -Amylase inhibition activity

The sample solutions were mixed with 100 μL of 0.02 mol/L sodium phosphate buffer (pH 6.9) and 100 μL of α -amylase solution (4.5 units/mL/min) and pre-incubated at 25 °C for 10 min. Then, 100 μL of 1% starch solution was added and incubated at 25 °C for 30 min and the reaction was stopped by the addition of 1.0 mL dinitrosalicylic acid. The test tubes were then incubated

in a boiling-water bath for 5 min and then cooled to room temperature. The reaction mixture was then diluted 10-fold times with distilled water, and the absorbance was measured at 540 nm [16]. The readings were compared to the control (the extract was replaced by sodium phosphate buffer) and α -amylase inhibition activity (%) was calculated as:

$$\alpha\text{-amylase inhibition activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control at 540 nm and A_1 is the absorbance of the tested sample at 540 nm.

2.5.2. α -Glucosidase inhibition activity

The sample solutions were mixed with 100 μ L of 0.1 mol/L phosphate buffer (pH 6.9) and 100 μ L α -glucosidase solution (1 unit/mL/min) and incubated at 25 °C for 5 min. After the pre-incubation, 100 μ L of *p*-nitrophenyl- α -D-glucopyranoside (5 mmol/L) solution was added, and the reaction mixture was incubated at 25 °C for 10 min. After the incubation, the absorbance was recorded at 405 nm and α -glucosidase inhibition was calculated as [16]:

$$\alpha\text{-glucosidase inhibition activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the tested sample.

2.5.3. Tyrosinase inhibitory activity

Tyrosinase inhibitory activity was determined according to the modified method of Chang et al. [17]. 20 μ L tyrosinase (1000 U/mL) was mixed with 100 μ L sample solution (dissolved in DMSO) and 1.9 mL of 50 mmol/L phosphate buffer (pH 6.8). The reaction mixture was incubated at 25 °C for 5 min. Then, 880 μ L L-DOPA as a substrate was added to start the reaction. The increase in absorbance was recorded at 475 nm. Kojic acid was used as a positive control. The tyrosinase inhibitory activity was calculated as:

$$\text{Tyrosinase inhibitory activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the tested sample.

2.5.4. Acetylcholinesterase inhibitory activity

Acetylcholinesterase (AChE) inhibitory activity was determined according to the modified method of Ellman et al. [18]. A 300 μ L of 100 mmol/L sodium phosphate buffer (pH 8.0), 10 μ L sample solution and 40 μ L AChE (5.32×10^{-3} U) solution were mixed and incubated at 25 °C for 15 min and added to 20 μ L of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (0.5 mmol/L). Then the reaction was initiated by adding 20 μ L acetylthiocholine iodide (0.71 mmol/L). The absorbance of the reaction mixture was read at 412 nm. Eserine was used as a reference compound. The inhibition of AChE was calculated by using $[(E - S)/E] \times 100$, where E is the activity of enzyme without the test sample and S is the activity of enzyme with the test sample.

2.6. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA), and the significance was determined by Duncan's multiple-range test ($P < 0.05$) using SPSS 13. Values expressed are means of triplicate determinations \pm standard deviation ($n = 3$). Pearson's correlation test was conducted to determine the linear correlations among variables.

3. Results and discussion

3.1. Functional properties of *Z. mauritiana* mucilage

Hydration properties of mucilage refer to its ability to retain water within the matrix. Fiber with strong hydration properties will increase stool weight and its viscosity properties impedes absorption of macronutrients resulting in increased insulin sensitivity, increased satiety and decreased energy intake. The WHC of crude ZMM was found to be 25.21 g/g (Table 1). The WHC of crude ZMM is higher than those reported in DF of some fruits (e.g. apple, pear, banana, lime peel and bambangan peel) [19,21] which is in the range of 6.3–12.8 g/g. But in comparison with pitaya peels (54.20 g/g) [22] WHC of ZMM is found to be lower. The ability to increase in bulk after absorbing water is one of the important functional properties of fibers. The SC of crude ZMM (19.34 mL/g) is similar to that of coconut (18.3 mL/g) and mango fiber (18.7 mL/g), but it is lower than that of pitaya (35.95 mL/g) and carrot (60.2 mL/g) DF [19,22]. It has been suggested that WHC and SC might possibly be affected by the amount of hydrophobic polysaccharide like soluble DF, which is contained within the cell wall of plants [23]. Therefore, the high amount of soluble DF in crude ZMM possessed WHC and SC through associating with absorption of large amounts of water. The high WHC of soluble fiber has an effect on their viscosity; the viscous fiber in the intestinal content reduces the absorption of glucose in the gut which helps to reduce the postprandial blood-glucose level.

DF is able to retain oil thus able to absorb/bind bile acids and increase their excretion which is associated with plasma cholesterol reduction. The OHC of crude ZMM (12.53 g/g, Table 1) is much higher than that of other reported DF such as orange, mango, pitaya fiber, which is in range of 1.27–4.65 g/g [21,24]. OHC depends on the surface properties of fiber, thickness and the hydrophobic nature of the fiber particle. The exact nature of the interaction between the bile acids and dietary fiber is still unclear to date. It may be due to the hydrophobic nature of DF, which form intraluminal micelle and reduces cholesterol content in plasma, leading to up regulation of LDL receptors and increased clearance of LDL cholesterol [25].

EC is a molecule's ability to act as an agent that facilitates solubilization or dispersion of two immiscible liquids and ES is the ability to maintain an emulsion and its resistance to rupture. EC and ES of crude ZMM were found to be 54.12% and 42.14%, respectively (Table 1). Mucilage has effective EC via interfacial absorption and the subsequent formulation of condensed films with high-tensile strength that resist coalescence of droplets. Furthermore, it stabilizes oil/water emulsions by

Table 1
Functional properties of ZMM.

Parameters	Value
WHC (g/g)	25.21 ± 2.34
OHC (g/g)	12.53 ± 1.45
Swelling capacity (mL/g)	19.34 ± 1.32
Emulsifying capacity (%)	54.12 ± 1.67
Emulsifying stability (%)	42.14 ± 1.09

Note: ZMM, *Z. mauritiana* mucilage; WHC, water holding capacity; OHC, oil holding capacity. Similarly herein after.

forming a strong multimolecular film around each oil globule and thus retards the coalescence by the presence of a hydrophilic barrier between the oil and water phases [26]. The EC of fiber is very indicative in health potential benefits. Since it absorbs biliar acids, absorption of these acids in the small intestine and excretion in the feces helps to reduce blood cholesterol levels [27]. Therefore, intake of *Z. mauritiana* fruit enriched in fiber might be helpful to reduce hypercholesterolemic effects (Table 1).

3.1.1. Glucose absorption capacity

The effect of dietary fiber in the absorption of the glucose at different concentrations (10–200 mmol/L) was investigated. This value predicts the behavior of fiber on absorbing the glucose in the gastrointestinal tract. Table 2 reveals that crude ZMM, xanthan and guar gum could bind glucose (0.15–0.97 to 15.34–19.80 mmol/g) at different concentrations (10–200 mmol/L) effectively, and the amount of glucose bound to these dietary fibers were concentration dependent. The absorption of trace amount of the glucose (10 mmol/L) attributes to the fact that crude ZMM could keep the glucose in the intestinal lumen even at low concentrations. In general, the glucose absorption capacity of crude ZMM was found to be lower than that of xanthan and guar gum.

3.1.2. Effect on glucose diffusion and glucose dialysis retardation index (GDRI)

The effect of various samples on the movement of glucose across the dialysis membrane is presented in Table 3. The diffusion of glucose was time-dependent, and higher amounts of glucose were found in the dialysate with the increasing of time from 30 to 120 min. The glucose content in the dialysate of crude ZMM, xanthan gum and guar gum were elevated from 165.0 to 329.11 μmol , 153.0 to 215.54 μmol , and 160.3 to 224.43 μmol respectively, as the time increased from 30 to 120 min. Compared with the control (190.05–412.11 μmol), all the samples significantly reduced the content of diffused glucose. Based on the retardation of glucose diffusion in the dialysis membrane, GDRI for all the samples was obtained (Table 4). GDRI is a useful *in vitro* index to predict the effect of fiber on the delay of glucose absorption in the gastrointestinal tract [28]. The GDRI of crude ZMM at 30 min was 37.07%; as the time increased from 60 to 120 min, GDRI generally was diminished, but the retarding effect of crude ZMM in all the time duration was found to be lower compared with that of the xanthan and guar gum. The GDRI value of crude ZMM at 60 min in this study was lower

than those reported for mango peel, apple pectin and bambangan peel fiber [19]. The mechanism of action in polysaccharides for glucose reduction is mainly due to the formation of viscous gel in aqueous solution by soluble fiber. Viscosity reduces the accessibility of glucose to the absorptive epithelium in the small intestine, thereby blunting postprandial glucose level. This phenomenon reduces the diffusion rate of glucose and may have a potential benefit to control postprandial blood glucose.

3.1.3. Effect of dietary fiber on diffusion of glucose in a starch- α -amylase-dietary fiber system

In humans, starch and its derivatives are digested in several stages. In the mouth, in contact with saliva α -amylase, the starch polymeric chains are cleaved into shorter oligosaccharides. Upon entering the gut, the partially digested material is further hydrolyzed by human pancreatic α -amylase. The main resulting products, maltose and branched dextrins, are converted into glucose by the brush border maltose-glucoamylase and sucrase-isomaltase and then enter the blood stream. Thus plasma glucose level increases about 10 min after a meal containing carbohydrates. We have examined the effect of crude ZMM on the activity of α -amylase to hydrolyze starch by measuring the amount of glucose in dialysate (Table 4). The diffused glucose content in the dialysate of crude ZMM, xanthan and guar gum were elevated from 110.01 to 378.65 μmol , 129.12 to 441.64 μmol and 101.16 to 431.39 μmol respectively, as the time increased from 20 to 180 min. Compared with the control (333.16–866.23 μmol), all the samples significantly reduced the content of diffused glucose. The crude ZMM has reduced the glucose diffusion. However in comparison with the xanthan and guar gum, the crude ZMM showed lower glucose diffusion at all time intervals. The retardation of glucose diffusion is also due to the inhibition of α -amylase, thereby limiting the release of glucose from the starch. The inhibition of α -amylase activity might be attributed to several factors such as fiber concentration, the presence of inhibitors in fibers, encapsulation of starch and enzyme by the fibers present in the sample. These may reduce the accessibility of starch to the enzyme and direct adsorption of the enzyme on fibers, leading to decreased amylase activity [3]. Inhibitors of carbohydrate hydrolyzing enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise. These observations emphasize that inhibition of α -amylase is one of the probable mechanisms through which the samples exert its hypoglycemic effect. Further studies are needed to investigate whether the DF consist inhibitors of α -amylase or simply act as a barrier between enzyme and starch.

3.1.4. FT-IR study and SEM examination of ZMM

FT-IR spectroscopy has been shown to be a useful tool in monitoring structural characteristics. The FT-IR spectrum of crude ZMM is shown in Fig. 1. The FT-IR spectra of ZMM showed broad O–H stretching at 3365 cm^{-1} . A short peak observed at 2918 cm^{-1} corresponds to antisymmetric- CH_2 . The sample exhibited the characteristic's IR absorption of polysaccharides at 1706 cm^{-1} , which corresponds to carbonyl groups

Table 2
Glucose-adsorption capacity of ZMM, xanthan gum and guar gum at different glucose concentrations.

Samples	Glucose bound (mmol/g)				
	200 mmol/L	100 mmol/L	50 mmol/L	25 mmol/L	10 mmol/L
ZMM	15.34 ± 1.21	7.32 ± 1.03	3.12 ± 0.09	1.11 ± 0.18	0.15 ± 0.00
Xanthan gum	19.80 ± 1.01	9.84 ± 1.01	4.88 ± 0.30	2.44 ± 0.00	0.97 ± 0.00
Guar gum	19.77 ± 1.01	9.81 ± 1.04	4.87 ± 0.32	2.43 ± 0.00	0.97 ± 0.00

Table 3
Effect of ZMM, guar gum and xanthan gum on glucose diffusion and GDRI.

Samples	30 min		60 min		90 min		120 min	
	Dialyzed glucose (μmol)	GDRI (%)	Dialyzed glucose (μmol)	GDRI (%)	Dialyzed glucose (μmol)	GDRI (%)	Dialyzed glucose (μmol)	GDRI (%)
Control	190.05 ^a ± 1.2	0	280.65 ^a ± 1.4	0	301.32 ^a ± 1.9	0	412.11 ^a ± 1.8	0
ZMM	165.0 ^b ± 1.5	15.7 ^b ± 1.5	174.09 ^c ± 1.7	37.07 ^a ± 1.0	215.09 ^b ± 1.6	29.13 ^a ± 1.5	329.11 ^b ± 1.5	21.14 ^a ± 1.1
Xanthan gum	153.0 ^d ± 1.5	22.45 ^a ± 1.0	160.08 ^d ± 1.9	44.58 ^a ± 0.8	194.14 ^c ± 1.4	38.15 ^b ± 1.9	215.54 ^d ± 1.5	47.12 ^b ± 1.2
Guar gum	160.3 ^c ± 1.9	17.12 ^b ± 1.0	179.13 ^b ± 1.4	25.13 ^b ± 0.2	185.14 ^d ± 1.2	18.10 ^c ± 0.9	224.43 ^c ± 1.4	46.04 ^b ± 1.8

Note: GDRI, glucose dialysis retardation index. Mean values followed by different letters in a column are significantly different ($P < 0.05$). Similarly herein after.

Table 4
Effect of mucilage on diffusion of glucose in a starch- α -amylase-DF system.

Samples	Glucose dialysate (μmol)				
	20 min	30 min	60 min	120 min	180 min
Control	333.16 ^a ± 0.34	372.84 ^a ± 0.18	498.52 ^a ± 0.16	609.04 ^a ± 0.18	866.23 ^a ± 0.29
ZMM	110.01 ^c ± 1.99	143.43 ^d ± 1.14	197.56 ^d ± 0.95	284.83 ^d ± 0.58	378.65 ^d ± 1.23
Xanthan gum	129.12 ^b ± 0.22	178.07 ^b ± 0.32	246.45 ^b ± 0.32	370.69 ^b ± 0.15	441.64 ^b ± 0.19
Guar gum	101.16 ^c ± 0.18	153.49 ^c ± 0.19	221.81 ^c ± 0.42	355.81 ^c ± 0.12	431.39 ^c ± 0.13

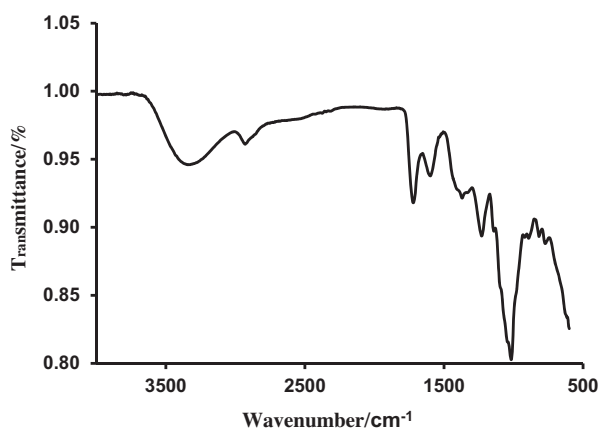


Fig. 1. FT-IR spectrum of ZMM.

(C=O). The aromatic C=C stretching appeared at 1583 cm^{-1} and C–O stretching of phenolic OH and aryl methyl esters appeared at 1218 cm^{-1} . Combination of C–O stretching and OH deformation appeared at 1010 cm^{-1} , which is a fingerprint region for all the fiber samples. Based on this information, we proposed that the high WHC and OHC of mucilage may be due to the presence of hydroxyl groups.

The morphological characteristic of crude ZMM was visualized by SEM and shown in Fig. 2. It showed more irregular

and uneven surfaces with large number of cracks. The available surface and the regiochemistry of the surface layer might play a role in some physicochemical properties (adsorption or binding of some molecules) accounting for some physiological effects DF [29].

3.2. Total phenolic content and antioxidant activity of ZMM

Phenolic compounds are major contributors of the antioxidant capacity of fruits and vegetables. Polyphenols associated with polysaccharides behave as DF constituents. Bioactive compounds (polyphenols) in the polysaccharides have been shown to be protective against various diseases like atherosclerosis, diabetes and neurodegenerative disorders mostly through their antioxidant and free radical scavenging capacities. The total phenolic content of crude ZMM was 25.54 mg/g (Table 5), which was higher than that of bambangan peels (98.3 mg/g) [19], Hayden mango peels (70 mg/g) [30] and Indian mango peel (96.2 mg/g) [20], but similar to that of unripe bambangan peels (24 mg/g) [31]. The phenolic compounds in the crude ZMM possessed potential antioxidant activity (Table 2). The value of DPPH and ABTS free radical was expressed as IC_{50} g DPPH/g mucilage and mmol trolox equiv./g mucilage and both the assays were compared with BHA. A lower IC_{50} value reflects a higher scavenging activity. In scavenging the DPPH \cdot and ABTS $^{+\cdot}$, BHA (0.15 g DPPH/g extract; 654,356.06 mmol trolox

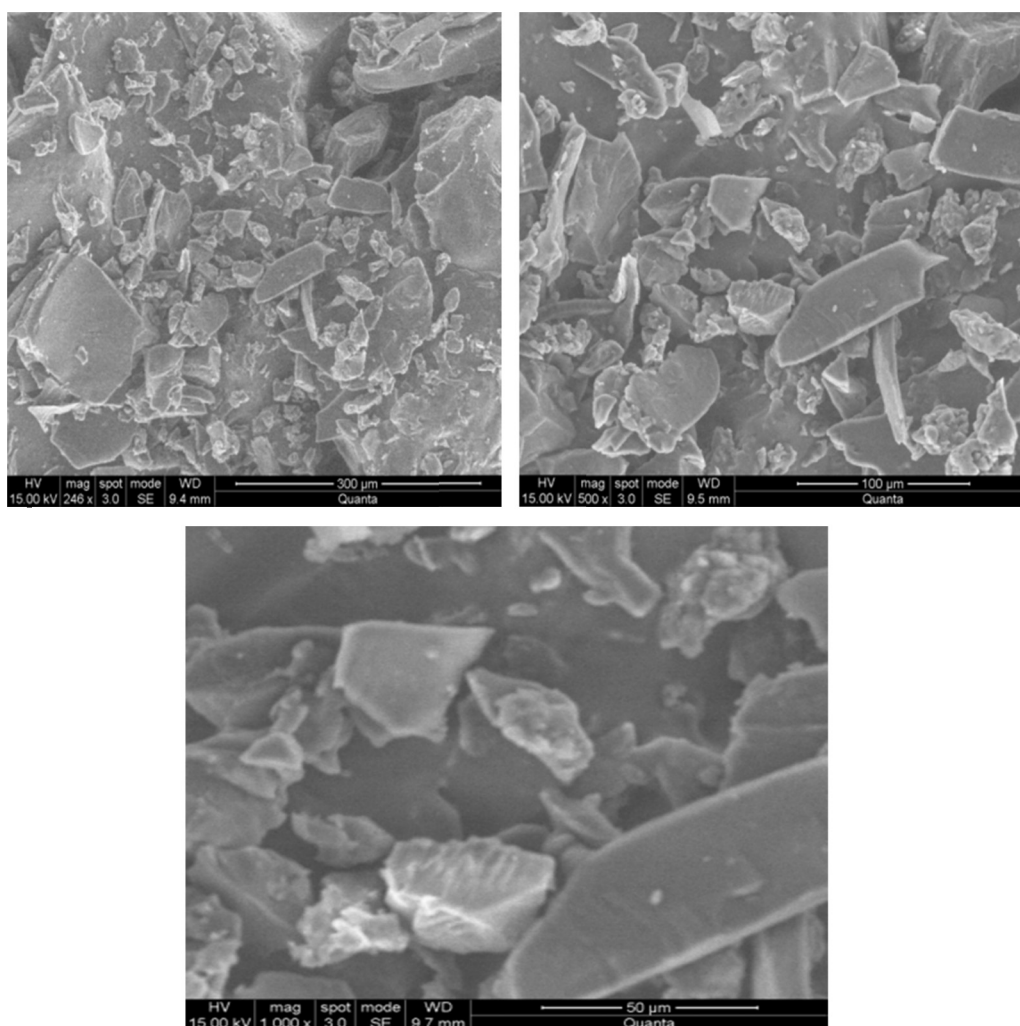


Fig. 2. Scanning electron micrograph of ZMM.

equiv./g) exhibited higher radical scavenging activity than the crude ZMM (5.27 g DPPH/g extract; 16,587.32 mmol trolox equiv./g mucilage). Thus, the results indicated that ZMM could also effectively react with DPPH[•] and ABTS^{•+} to convert them to more stable products by donating the proton and terminating the radical chain reaction.

The superoxide and hydroxyl radical are the powerful oxidizing agents that can react with biological membranes and induce tissue damages. The scavenging capability of these radicals by crude ZMM is directly related to its antioxidant activity

(Table 2). The superoxide and hydroxyl radical scavenging ability of crude ZMM was found to be 85.12% and 76.13%, which is higher than that of the previously reported *Physalis alkekengi* fruit polysaccharides (17.1%; 21.6%) [32]. Compared with the positive control catechin, catechin exhibited higher radical scavenging activity than the crude ZMM. The phenolic compounds in the crude ZMM possessed antioxidant capability toward scavenging DPPH, hydroxyl and super oxide radical; hence, the present study points out crude ZMM as an important antioxidant DF source.

Table 5

Total phenolics, DPPH radical, ABTS cation radical, superoxide and hydroxyl radical scavenging activity of ZMM.

Samples	Total phenolics (mg GAE/g mucilage)	DPPH ^a (IC ₅₀ g mucilage/g DPPH)	TEAC ^b (mmol trolox equiv./g)	Super oxide radical scavenging activity (%)	Hydroxyl radical scavenging activity (%)
ZMM	25.54 ± 1.1	5.27 ± 0.05	16,587.32 ± 29.9	85.12 ± 1.7	76.13 ± 2.3
BHA	–	0.15 ± 0.01	654,356.06 ± 617.13	–	–
CAT	–	–	–	94.08 ± 1.5	82.46 ± 2.2

Note: BHA, butylated hydroxyanisole; CAT, catechin.

^a Gram mucilage required to decrease one g of the initial DPPH concentration by 50%.

^b Trolox equivalent antioxidant capacity (TEAC) (mmol equivalent trolox performed by using ABTS^{•+} radical cation).

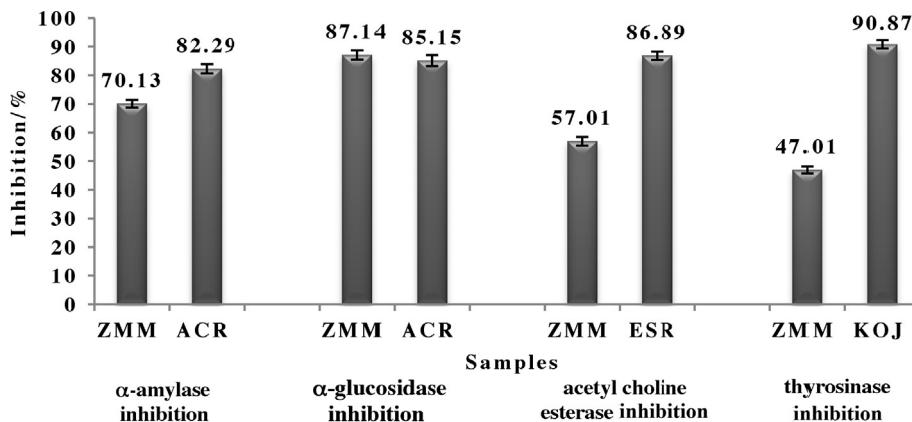


Fig. 3. Effect of α -amylase, α -glucosidase, acetylcholine esterase and tyrosinase enzyme inhibitory activity by ZMM. Note: ZMM; ACR, acarbose; ESR, eserine; KOJ, kojic acid.

3.3. Effect of ZMM on inhibition of various enzymes *in vitro*

Earlier reports have revealed that oxidative injury plays a main function in pathogenesis of many diseases, including diabetes, Alzheimer's disease and skin pigmentation. Besides, oxidation may also change the nutritional quality of food. The crude ZMM, with the presence of polyphenols, inhibits the enzymes responsible for above-mentioned facts. The inhibitory activity of α -amylase, α -glucosidase, acetylcholine esterase and tyrosinase enzymes is shown in Fig. 3. Inhibition of enzymes involved in the hydrolysis of carbohydrates such as α -amylase and α -glucosidase have been exploited as a therapeutic approach in controlling postprandial hyperglycemia. Pancreatic α -amylase is involved in the breakdown of starch into disaccharides and oligosaccharides before intestinal α -glucosidase catalyzes the breakdown of disaccharides to liberate glucose, which is later absorbed from the blood circulation. Inhibition of these enzymes would slow down the breakdown of starch in the gastro-intestinal tract, thus reducing postprandial hyperglycemia [33]. The α -amylase and α -glucosidase inhibitory activity of crude ZMM was compared with the control acarbose. The inhibitory effect of crude ZMM on α -amylase and α -glucosidase was 70.13% and 87.14%, respectively, at the concentration of 250 μ g/mL. The inhibitory activity of acarbose is high in α -amylase activity, but low for α -glucosidase in comparison with the crude ZMM. Our results were in agreement with those of previous studies, which have demonstrated that plant-based phenolic phytochemicals have lower α -amylase inhibitory action but stronger inhibitory potential against α -glucosidase [34], and such natural enzyme inhibitors is likely to offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia, due to lower abdominal side effects arising from excessive inhibition of pancreatic α -amylase by synthetic drugs (acarbose, miglitol and metformin), which results in the abnormal bacterial fermentation of undigested carbohydrates in the colon. Therefore, these results indicate that crude ZMM rich in phenolics have the potential in contributing to the management of type II diabetes.

Tyrosinase, a well-known polyphenol oxidase, widely existing in plants and humans, is responsible for the biosynthesis

of melanin. Tyrosinase (binuclear copper containing enzyme), converts the tyrosine to DOPA quinone through monophenolase and diphenolase, which polymerizes to form melanin. Melanin determines the actual color of skin, and it protects skin from UV radiation. The reactive quinone mixes with the polyphenolic substrates in the presence of oxygen through nonenzymatic coupling and spontaneously evolves to form an enzymatic browning in foods and hyper pigmentation in humans. Enzymatic browning affects the color, flavor and nutritional quality of food and hyperpigmentation affects the skin health of humans. Therefore, the development of natural tyrosinase inhibitor is an important alternative approach in maintaining the food quality and skin health. The inhibitory activity of crude ZMM compared with kojic acid (positive control) was evaluated. Although the tyrosinase inhibitory activity of crude ZMM (47.01%) is lower than that of the kojic acid (90.87%), it is still able to increase the anti-tyrosinase activity due to its antioxidant power. Tyrosinase inhibitors usually chelate with copper within the tyrosinase active site thereby obstructing the substrate–enzyme interaction, or prevent oxidation via an electrochemical process [35]. However, the presence of the hydroxyl group in crude ZMM may form hydrogen bond with the active site of enzyme and inhibit the tyrosinase activity. Therefore, crude ZMM may be applied in medicinal, cosmetic and food industry to maintain the skin health and food quality.

The ACh is one of the major compounds by which nerve impulses are transmitted from a neuron to neuron or involuntary muscle. The function of ACh in the nerve impulse transmission is terminated by AChE at the cholinergic synapses by rapid hydrolysis of ACh to choline and acetate. This results in a strategy of treating Alzheimer's disease, senile dementia, ataxia, myasthenia gravis and Parkinson's disease [36]. Few synthetic medicines (e.g. acrine, donepezil) and the natural product-based rivastigmine are used in the treatment of cognitive dysfunction and memory loss associated with Alzheimer's disease. These compounds have been reported to have their adverse effects, including gastrointestinal disturbances and problems associated with bioavailability [37], which necessitates the interest in finding better AChE inhibitors from natural resources. The interaction of crude ZMM and eserine (positive control) with AChE

was evaluated. The results reveal that crude ZMM inhibited the AChE (57.01%), but it was lower compared with eserine (86.89%). It could be postulated that AChE inhibition activity of crude ZMM might be due to phenolic compounds.

4. Conclusion

The crude mucilage isolated from the *Z. mauritiana* fruit acts as a nutraceutical product through various physiological effects. The role of crude ZMM in controlling the postprandial serum glucose level may be illustrated by three mechanisms. Firstly, ZMM with good WHC and SC, increases the viscosity of small intestine content and hinders the diffusion of glucose. Secondly, glucose binds with the mucilage and decreases the amount of available glucose in the small intestine with high GDRI. Thirdly, it retards the α -amylase and α -glucosidase action and postpones the release of glucose due to the presence of fiber associated total polyphenols. In addition, ZMM exhibited strong antioxidant capacity through scavenging DPPH, ABTS, hydroxyl and superoxide radicals. Besides, promising AChE and tyrosinase inhibitory action reveals that mucilage also has a capability to control the Alzheimer's disease, skin diseases and food spoilage. Therefore, the novel crude mucilaginous fraction isolated from *Z. mauritiana* fruit is an excellent source toward its development as a potential nutraceutical/functional food in the prevention and control of diabetes, Alzheimer's disease and skin disease. Furthermore, it is also important to study the detailed structure and conformation of the above-mentioned polysaccharides and their impacts on health effects through *in vivo* experiments.

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References

- [1] A.R. Bird, A. Lopez-Rubio, A.K. Shrestha, et al., Resistant starch *in vitro* and *in vivo*: factors determining yield, structure, and physiological relevance, in: S. Kasapis, I.T. Norton, J.B. Ubbink (Eds.), *Modern Biopolymer Science: Bridging the Divide between Fundamental Treatise and Industrial Application*, Academic Press, Burlington, MA, 2009, pp. 6–15 (Chapter 14).
- [2] F. Figuerola, M.L. Hurtado, A.M. Estévez, et al., Fiber concentrates from apple pomace and citrus peel as potential fiber sources for food enrichment, *Food Chem.* 91 (2005) 395–401.
- [3] S. Ou, K.C. Kwok, Y. Li, et al., *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose, *J. Agric. Food Chem.* 49 (2001) 1026–1029.
- [4] L.K. Nyanga, T.H. Gadaga, M.J.R. Nout, et al., Nutritive value of masau (*Zizyphus mauritiana*) fruits from Zambezi valley in Zimbabwe, *Food Chem.* 138 (2013) 168–172.
- [5] T.K. Koley, C. Kaur, S. Nagal, et al., Antioxidant and phenolic content in genotypes of Indian jujube (*Zizyphus mauritiana* Lamk), *Arabian J. Chem.* 4 (2011) 1–9.
- [6] J. Li, L. Shana, J. Liu, et al., Screening of a functional polysaccharide from *Zizyphus jujuba* cv. Jinsixiaozao and its property, *Int. J. Biol. Macromol.* 49 (2011) 255–259.
- [7] J. Li, L. Ai, Q. Yang, et al., Isolation and structural characterization of a polysaccharide from fruits of *Zizyphus jujuba* cv. Junzao, *Int. J. Biol. Macromol.* 55 (2013) 83–87.
- [8] D. Wang, Y. Zhao, Y. Jiao, et al., Antioxidative and hepatoprotective effects of the polysaccharides from *Zizyphus jujube* cv. Shaanbeitanzao, *Carbohydr. Polym.* 88 (2012) 1453–1459.
- [9] J.A. Robertson, F.D. de Monredon, P. Dysseler, et al., Hydration properties of dietary fiber and resistant starch: a European collaborative study, *LWT-Food Sci. Technol.* 33 (2000) 72–79.
- [10] V.Q. Neto, N. Narain, J.B. Silvia, et al., Functional properties of raw and heat-processed cashew nut (*Anacardium occidentale* L.) kernel protein isolate, *Nahrung* 45 (2001) 258–262.
- [11] P. Siddhuraju, K. Becker, Antioxidant properties of various extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves, *J. Agric. Food Chem.* 51 (2003) 2144–2155.
- [12] C. Sanchez-Moreno, J.A. Larrauri, F. Saura-Calixto, Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents, *Food Res. Int.* 32 (1999) 407–412.
- [13] R. Re, N. Pellegirini, A. Progettente, et al., Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.* 26 (1999) 1231–1237.
- [14] S.M. Klein, G. Cohen, A.I. Cederbaum, Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical-generating systems, *Biochemistry* 20 (1991) 6006–6012.
- [15] A.C. Martinez, E.L. Marcelo, A.O. Marco, et al., Differential responses of superoxide dismutase in freezing resistant *Solanum curtibolum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress, *Plant Sci.* 160 (2001) 505–515.
- [16] V. Vadivel, H.K. Biesalski, Contribution of phenolic compounds to the antioxidant potential and type II diabetes related enzyme inhibition properties of *Pongamia pinnata* L. Pierre seeds, *Process Biochem.* 46 (2011) 1973–1980.
- [17] E.W.C. Chang, Y.Y. Lim, L.F. Wong, et al., Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species, *Food Chem.* 109 (2008) 477–483.
- [18] G.L. Ellman, K.D. Courtney, V. Andres, et al., A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* 7 (1961) 88–95.
- [19] F.A. Hassan, A. Ismail, A.A. Hamid, et al., Characterisation of fibre-rich powder and antioxidant capacity of *Mangifera pajang* K. fruit peels, *Food Chem.* 126 (2011) 283–288.
- [20] C.M. Ajila, M. Aalami, K. Leelavathi, et al., Mango peel powder: a potential source of antioxidant and dietary fiber in macaroni preparations, *Innov. Food Sci. Emerg. Technol.* 11 (2010) 219–224.
- [21] S.H. Al-Sheraji, A. Ismail, M.Y. Manap, et al., Functional properties and characterization of dietary fiber from *Mangifera pajang* Kort. fruit pulp, *J. Agric. Food Chem.* 59 (2011) 3980–3985.
- [22] Y. Zhuang, Y. Zhang, L. Sun, Characteristics of fiber-rich powder and antioxidant activity of pitaya (*Hylocereus undatus*) peels, *Int. J. Food Sci. Technol.* 47 (2012) 1279–1285.
- [23] C. Sáenz, E. Sepúlveda, B. Matsushiro, *Opuntia* spp. mucilage's: a functional component with industrial perspectives, *J. Arid Environ.* 57 (2004) 275–290.
- [24] M. Elleuch, D. Bedigian, O. Roiseux, et al., Dietary fiber and fiber-rich by-products of food processing: characterisation, technological functionality and commercial applications: a review, *Food Chem.* 124 (2011) 411–421.
- [25] J.W. Anderson, J.T. Tietyen-Clark, Dietary fiber: hyperlipidemia, hypertension and coronary artery diseases, *Am. J. Gastroenterol.* 81 (1986) 907–919.
- [26] V.D. Prajapati, G.K. Jain, N.G. Moradiya, et al., Pharmaceutical applications of various gums, mucilages and their modified forms, in: C.J. Bailey (Ed.), *Textbook of Diabetes*, Blackwell Science, Oxford, 2003, pp. 1–73.
- [27] G. Lopez, G. Ros, F. Rincon, et al., Relationship between physical and hydration properties of soluble and insoluble fiber of artichoke, *J. Agric. Food Chem.* 44 (1996) 2773–2778.

- [28] S.Y. Ou, K.R. Gao, Y. Li, An *in vitro* study of wheat bran binding capacity for Hg, Cd, and Pb, *J. Agric. Food Chem.* 47 (1999) 4714–4717.
- [29] F. Guillon, A. Auffret, J.A. Robertson, et al., Relationships between physical characteristics of sugar beet fibre and its fermentability by human faecal flora, *Carbohydr. Polym.* 37 (1998) 185–197.
- [30] J.A. Larrauri, P. Rupérez, B. Borroto, et al., Mango peels as a new tropical fiber: preparation and characterization, *LWT-Food Sci. Technol.* 29 (1996) 729–733.
- [31] M.F. Abu Bakar, M. Mohamed, A. Rahmat, et al., Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*), *Food Chem.* 113 (2009) 479–483.
- [32] Y. Ge, Y. Duan, G. Fang, et al., Study on biological activities of *Physalis alkekengi* var. *francheti* polysaccharide, *J. Sci. Food Agric.* 89 (2009) 1593–1598.
- [33] Y.I. Kwon, E. Apostolidis, Y.C. Kim, et al., Health benefits of traditional corn, beans and pumpkin: *in vitro* studies for hyperglycemia and hypertension management, *J. Med. Food* 10 (2007) 266–275.
- [34] E. Apostolidis, C. Lee, *In vitro* potential of *Ascophyllum nodosum* phenolic antioxidant-mediated α -glucosidase and α -amylase inhibition, *J. Food Sci.* 75 (2010) 97–102.
- [35] R. Matsuura, H. Ukeda, M. Sawamura, Tyrosinase inhibitory activity of citrus essential oils, *J. Agric. Food Chem.* 54 (2006) 2309–2313.
- [36] A.U. Rahman, M.I. Choudhary, et al., Bioactive natural products as a potential source of new pharmacophores: a theory of memory, *Pure Appl. Chem.* 73 (2001) 555–560.
- [37] V. Schulz, Ginkgo extract or cholinesterase inhibitors in patients with dementia: what clinical trial and guidelines fail to consider, *Phytomedicine* 10 (2003) 74–79.