ASM Sc. J., 13, Special Issue 6, 2020 for InCOMR2019, 53-59

Biofunctional Characteristics of Dietary Fibre from Malaysian *Ziziphus mauritiana* Leaves

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Ziziphus mauritiana derived from Rhamnaceae family plant (known as Bidara tree in Malaysia) has been consumed by Malaysian through processing the fruit into pickles or eating it fresh, while the leaves are commonly used in traditional medicine especially in Islamic medicine. However, *Z. mauritiana* plants are still underutilized in Malaysia as a lack of scientific information about its health promoting effect. Hence the study was aimed to investigate several properties related to its nutritional quality of the new cheap sources of dietary fiber (mucilage) which is needed in developing countries to maintain population health, especially in controlling diabetes. The extraction yield of mucilage was investigated from *Z. mauritiana* fruit pulp and leaves. The higher mucilage yield was obtained from the leaves with 1.24%, while the mucilage from the pulp yielded 0.34%. The mucilage of *Z. mauritiana* leaves with good hydration properties of swelling capacity (6.867ml/g ± 0.231), water holding capacity (3.960g/g ± 0.200), oil holding capacity (0.507g/g ± 0.083) and its emulsifying properties including emulsifying activity (56.0% ± 4.00) and emulsifying stability (70.87 % ± 2.31) indicate that it may have the capability in controlling the diabetes. Fourier transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM) analysis revealed the structural characteristic of the extracted *Z. mauritiana* mucilage. These properties make the crude mucilaginous fraction from *Z. mauritiana* leaves a remarkable candidate as potential dietary fiber for functional food and nutraceutical.

Keywords: *Ziziphus mauritiana*; dietary fibre (mucilage); functional characteristics; antidiabetic

I. INTRODUCTION

In recent years, there is such an interest in finding alternative therapy especially using a natural product. The implement of medicinal and aromatic herbs for the remedies of illnesses is as old as mankind. Medicinal plants acquire an interest in the research centre considering that their unique importance through the protection of communities (Najafi *et al*., 2010). Traditional medicines all over the world encompass a wide variety of natural drugs for the treatment of symptomatologies associated with chronic disorders such as diabetes mellitus (Farzaei *et al.*, 2019). Diabetes mellitus consists of two types which are diabetes type I and diabetes type II. Diabetes type I is the condition where the body produces very little to none insulin caused by the autoimmune reaction which the

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body's immune system attacking the insulin produced beta cells in the pancreas gland. While, diabetes type II is the condition where the body is unable to respond to insulin or also known as insulin resistance.

Within numerous genus of the family *Rhamnaceae* members, *Ziziphus mauritiana* have been utilized for hundreds of years in folk remedies (Najafi, 2013). *Z. mauritiana* plants exhibit significant levels of a useful bioactive compound that is beneficial for human. This plant features perform numerous applications in traditional remedies for example; the dried ripe fruits pose moderate laxative and are implemented on cuts and ulcers, as well as are applied in pulmonary ailments and fevers. Meanwhile, the leaves are useful in treating liver problems, asthma, and fever. Besides that, the powdered root is dusted on injuries (Dahiru *et al.*, 2006). An earlier study has been reported that the physical features of dietary fiber developed from *Z. mauritiana* fruit are about health effect (Sangeethapriya *et al.*, 2014). Previously, *Z. mauritiana* leaves have been broadly utilized as hypoglycemic agents to diabetics in several areas in Turkey (Erenmemisoglu *et al.*, 1995).

The explanation of dietary fibre initially came out during 1953 (Hipsley, 1953) through the context of explaining the food elements from the cell walls of plants (Macagnan *et al.*, 2016). According to American Association of Cereal Chemists (2010), dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. AACC (2010) also pointed out dietary fibre includes polysaccharides such as cellulose and hemicellulose, oligosaccharides, and lignin, as well as attached plant substances such as waxes, cutin, and suberin and assists enhance effective physiological outcome consist of laxation, blood cholesterol attenuation, and blood glucose attenuation (Chiewchan, 2018). Fibre is composed of polysaccharide networks interconnected via different types of bonds, including hydrogen bonds, electrostatic and dipolar interactions, Van der Waals attractions, to form a fibre matrix, and these characteristics promote the hydration (Eastwood *et al.*, 1992). Hence, this research aimed to study the biofunctional characteristic of dietary fibre extracted from Malaysian *Z. mauritiana* leaves.

I. MATERIALS AND METHOD

A. Sample Preparation

Fruits and leaves of *Z. mauritiana* were supplied by Saliran Mampan Sdn. Bhd, located in Pahang, Malaysia. Samples were cleaned and all the debris were removed. The mucilage preparation was described by Sangeethapriya *et al.*, (2014) with slight modification. Cleaned fruits and leaves were separately blended with water at the ratio of 1:7 and were then centrifuged (JLA 16.25) at 3000 X g for 30 minutes. The supernatant from the centrifuge then was collected and mixed with absolute ethanol at a ratio of 1:2. Then precipitate obtained was dried using a freeze dryer to form the powder and stored in an airtight container for further usage.

B. Swelling Capacity

According to the method by Sangeethapriya *et al.* (2014) with slight modification, 0.25 g mucilage powder was precisely weighed into a calibrated cylinder (triplicate), the volume of solid was recorded (V1) and 5 mL distilled water was added. After mixing, the mixture was left to stand at room temperature for 18 hours and the bed volume was recorded (V2). Swelling capacity (SC) was expressed as millilitre per gram of mucilage (mL/g) and calculated as mentioned (Robertson *et al.*, 2000):

$$Swelling\ capacity\ (mL/g): \frac{V2-V1}{0.25}$$

C. Water Holding Capacity (WHC)

The water holding capacity was analysed according to a method done by Sangeethapriya *et al.* (2014). Five millilitres distilled water was added to 0.25 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 × g for 15 min, the supernatant was carefully discarded, and the residue was weighed (m). Water holding capacity (WHC) was expressed as the amount of water retained per gram of mucilage powder (g/g). The WHC was calculated as follows (Robertson *et al.*, 2000):

$$Water\ holding\ capacity\ (g/g): \frac{m - 0.25}{0.25}$$

D. Oil Holding Capacity (OHC)

The oil holding capacity was investigated according to Sangeethapriya *et al.*, (2014) method. Five millilitres refined sunflower oil was added to 0.25 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 × g for 15 min, the supernatant was carefully discarded, and the residue was weighed (m). Water holding capacity (OHC) was expressed as the amount of oil retained per gram of mucilage powder (g/g). The OHC was calculated as follows (Robertson *et al.*, 2000):

$$Oil\ holding\ capacity\ (g/g): \frac{m-0.25}{0.25}$$

E. Emulsifying Capacity (EC)

The mucilage emulsion capacity was determined by a method modified from Neto *et al.* (2001). Five millilitres of mucilage dispersion in distilled water (10mg/mL) was homogenized (1 min) with 5 mL sunflower oil. The emulsion was then centrifuged at 1100 X g for 5 min. Finally, the height of the emulsified layer was measured compared with the height of the whole layer and the emulsion capacity was measured by the following equation:

$$EC\ (\%): \frac{height\ of\ emulsified\ layer}{height\ of\ the\ whole\ layer}\ x\ 100$$

F. Emulsifying Stability (ES)

Emulsion stability was determined by heating the emulsion at 80°C for 30 min followed by centrifuging (1100 × g, 5 min) and calculated as follows:

$$ES\ (\%): \frac{\begin{matrix} height\ of\ the\ emulsified\ layer \\ after\ heating \end{matrix}}{\begin{matrix} height\ of\ emulsified\ layer \\ before\ heating \end{matrix}}\ x\ 100$$

G. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectra were recorded to identify the functional groups of the extracted mucilage with a Nicolet 6700 spectrometer (Thermo Fisher) in the spectral range of 4000–500 cm^{-1}. Samples were prepared by KBr pressed plate technique and the functional groups are determined from the graph.

H. Scanning Electron Microscopy (SEM)

The surface and microstructure of dietary fibres were observed using SEM (JSM 6400) at 15 kV. Powder samples were placed on double-sided conducting adhesive tapes and coated with a gold layer. Representative micrographs were taken for the sample at 3000X and 5000X magnification.

I. Statistical Analysis

The experimental data were analysed using the analysis of variance (ANOVA) and are presented as mean ± standard deviation (SD) of triplicates. The differences between mean values were established using Duncan's multiple range tests. Values were considered at a significance level of 95% ($p < 0.05$). Pearson's correlation test was conducted to determine the linear correlations among variables.

II. RESULT AND DISCUSSION

A. Mucilage Screening

Mucilage is a complex polysaccharide composed of several different monosaccharides and uronic acids (Chiewchan, 2018). The yield of extracted mucilage from *Z. mauritiana* leaves was found to present 0.82% on a dry weight basis (Table 1), which is comparatively higher than the extracted mucilage obtained from the fruits 0.34%. However, the yield of extracted mucilage obtained is lower than the previous report from other species of *Z. mauritiana* leaves and fruit pulp which is 7.10% and 3.97% (Clifford *et al.*, 2002; Thanatcha *et al.*, 2011). The differences in yield may be due to species variation, edaphic factors, extraction method and age of the plant (Souid *et al.*, 2011). Nuttall (1993), stated that the possibility that plant derived fibres may be useful in the management of blood glucose in individuals with Non-

Insulin Dependent Diabetes Mellitus (NIDDM) was first suggested in 1976 by Dr. James Anderson in United stated and Dr. David Jenkins in United Kingdom.

Table 1. Yield % of mucilage extracted from *Z. mauritiana*

Sample	Mass (mg/100 mg)	Yield %	Reference
Fruits pulp	0.340	0.34	This study
Leaves	0.824	0.82	
Fruit pulp	3.970	3.97	Thanatcha *et al.*, 2011
Leaves	7.100	7.10	Clifford *et al.*, 2002

B. Swelling Capacity

Swelling capacity can be assessed by the bed volume technique, determined by swelling the fibre in water, in a volumetric cylinder. The swelling capacity of extracted mucilage was 6.867 mL/g, comparable to previously reported values of tragacanth gum 8.5 mL/g (Emeje *et al.*, 2011) and lower than values of *Z. mauritiana* fruit (19.34 mL/g) (Sangeethapriya *et al.*, 2014). This could be attributed to various factors like the presence of hydroxyl groups, high galactose units present in the extracted polysaccharide and the extraction method used (Kalegowda *et al.*, 2017). The swelling capacity shows the characteristics of soluble fibre that reported to have a significant effect on the post meal glucose concentration (Nuttall, 1993). The swelling capacity is useful for modulating the drug release by improving the pore characteristics of the excipient (Khullar *et al.*, 1998). Thus, the isolated mucilage with high swelling property could be used as a disintegrating or binding agent in pharmacological industries.

C. Water Holding Capacity (WHC)

Water holding capacity is the amount of water that is retained by a known dry weight of fibre under specified conditions of temperature, soaking time, and speed of centrifugation (Fleury *et al.*, 1991). It was found that the water holding capacity of the mucilage powder was 3.96 g/g which was comparable to *Opuntia dillenii* cladode mucilage 4.0 g/g (Kalegowda *et al.*, 2017) but lower than that of mucilage from *Z. mauritiana* fruit 11.7g/g (Thanatcha *et al.*, 2011). The ability to increase in bulk after absorbing water is one of the important functional properties of the fibre. Miguel and Belloso (1999) stated that WHC represents the percentage of hydrophilic fraction, which has a greater affinity to absorb water. Dietary fibre with strong hydration properties will increase stool weight and its viscosity properties impede the absorption of macronutrients resulting in increased insulin sensitivity, increased satiety and decreased energy intake (Sangeethapriya and Siddhuraju, 2014). The forming of the viscous gel was due to the fibre that impaired with glucose absorption when added to water (Nuttall, 1993). Besides, the value of water holding capacity of *Z. mauritiana* mucilage can be related to the presence of polysaccharide in mucilage (Al-Sayed *et al.*, 2012).

D. Oil Holding Capacity

Oil holding capacity depends on the surface properties of fibre, thickness and the hydrophobic nature of the fibre particle (Sangeethapriya *et al.*, 2014). The oil holding capacity of the extracted mucilage from *Z. mauritiana* leaves obtained was 0.50 g/g which is lower than previously reported oil holding capacity of *Opuntia dillenii (Ker-Gawl) Haw* cladode mucilage 2 g/g (Kalegowda *et al.*, 2017) extracted mucilage from *Z. mauritiana* fruit of 4.96 g/g (Thanatcha *et al.*, 2011). The oil retention capacity is also been stated as a property that has physiological effects, since it can interfere in the intestinal fat absorption, influencing in the bodyweight control and the regulation of blood lipid profiles (Carvalho, 2009). The exact nature of the interaction between the bile acids and dietary fibre is still unclear to date (Sangeethapriya *et al.*, 2014).

Table 2: Functional properties of mucilage extracted from *Z. mauritiana* leaves

Functional Properties	Value
Swelling capacity (mL/g)	6.867 ± 0.231
Water holding capacity (g/g)	3.960 ± 0.200
Oil holding capacity (g/g)	0.507 ± 0.083
Emulsifying activity %	56.0 ± 4.00
Emulsion stability %	70.87 ± 2.31

E. Emulsifying Capacity

Emulsifying capacity is a molecule's potential in functioning as a mechanism that helps in solubilisation or distribution of two immiscible liquids, while emulsifying stability is the potential in preserving an emulsion and its resistance to break. Emulsifying capacity and emulsifying stability of mucilage leaves were discovered to be 56% and 70.87% respectively which were higher than previously reported mucilage obtained from the fruit pulp 54.12% and 42.14% (Sangeethapriya *et al.*, 2014) respectively.

Mucilage has powerful emulsifying capacity via interfacial absorption and the succeeding method of condensed films that has high-tensile strength which can withstand coalescence of droplets. Naturally, it stabilizes oil/water emulsions through creating a good multimolecular film surrounding every single oil parcelled and therefore remises those coalescences simply by the existence of a hydrophilic barrier in the middle of the oil and water phases (Sangeethapriya *et al.*, 2014). The emulsifying capacity of fibre is extremely indicative in wellbeing probable features. As emulsion absorbs bile acids, consumption of these kinds of acids through small intestine and excretion through feces allows in lessen blood cholesterol levels (López *et al.*, 1996).

F. Fourier transmitter infrared (FT-IR)

Infrared spectra of fibre from *Z. mauritiana* leaves show a strong peak at the peak of double bond C=O appear at 1617.96 cm^{-1} while triple bond considers as C≡O at 2361.96 cm^{-1} and C≡C at 2335.03 cm^{-1} wavenumber. The C-H stretch appears at wavenumber 2928.02 cm^{-1} meanwhile the hydroxyl group of O-H stretch can be seen at 3430.94 cm^{-1}. The presence of a peak at the single bond stretch that corresponded to the hydroxyl group which attributed to the properties of water holding capacity and oil holding capacity (Sangeethapriya *et al.*, 2014).

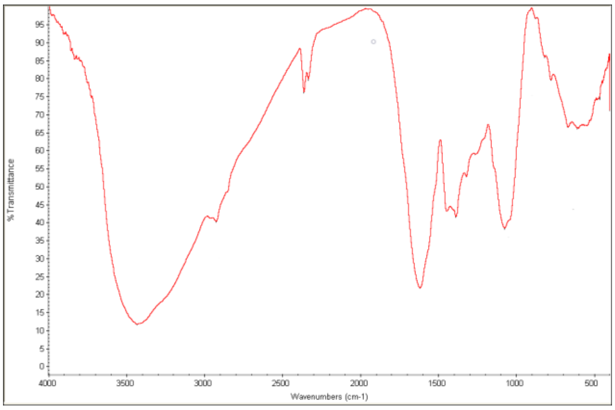
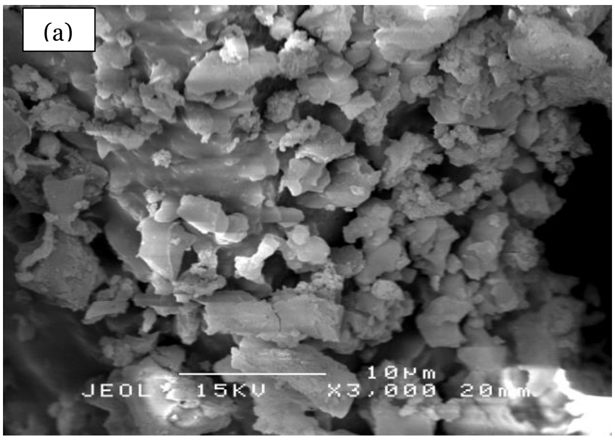


Figure 1. FT-IR spectrum of dietary fiber extracted from *Z. mauritiana* leaves

G. Scanning Electron Microscopic (SEM)

The microstructure of dietary fibre in *Z. mauritiana* leaves was observed using Scanning Electron Microscope (SEM) and the images were presented in micrograph as shown in figure 2 at a magnification of (a) 3000 X and (b) 5000 X. The microstructures of dietary fibre are related to their hydration, absorption and binding capacities such as the characteristic of porosity and available surface lead better adsorption properties (Elleuch *et al.*, 2011). Figure 2 displays the capillary structure of irregular surface with holes and the absence of starch granules. The holes or pores observed in the microstructure of dietary fibre may promote for easy diffusion of numerous components such as glucose and other sugars (Wang *et al.*, 2017) thus, helping to reduce those components that may cause of diabetes



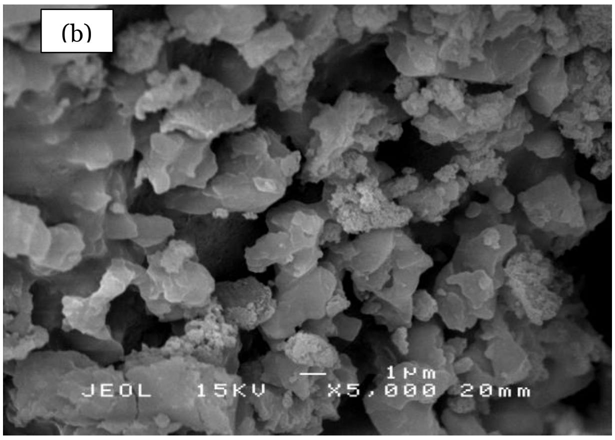


Figure 2. Scanning electron microscopy of dietary fibre extracted from *Z. mauritiana* leaves at magnification (a) 3000 X, and (b) 5000 X

III. CONCLUSION

The present study on the dietary fibre found in Malaysian *Z. mauritiana* leaves was higher than dietary fibre obtained in the fruit. The mucilage extracted from leaves of *Z. mauritiana* had performed good hydration properties in terms of swelling behaviour, water holding capacity, oil holding capacity and emulsifying properties.

The characteristic of microstructure for mucilage shows the possible way for diffusion of the glucose and sugar that are potential to reduce the indices of diabetes and obesity. Furthermore, the present study is the first report on the crude mucilaginous fraction extracted from Malaysian *Z. mauritiana* leaves demonstrating a conceivable source toward its development as a potential nutraceutical and functional food that can exhibit hampering effect on glucose diffusion resulting in lowered postprandial blood glucose.

IV. ACKNOWLEDGEMENTS

The authors acknowledge Food and Microbiome Laboratory (FAMTech), Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia to provide facilities and equipment. The study was supported by Putra Grant- Putra Graduate Initiative from Research Management Centre, Universiti Putra Malaysia.

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