

α -Glucosidase Inhibition and Biochemical Analysis of Extracts of *Ziziphus mauritiana* Young Leaves

Zalilawati Mat Rashid^{1, 2}, Nurrul Atikah Mohd Nasir¹, Natasha Aziz¹

¹Faculty of Bioresources and Food Industry,

²Institute of Agricultural Production and Food Innovation (AGROPOLIS),

Universiti Sultan Zainal Abidin, Besut Campus,

22200 Besut, Terengganu, Malaysia

Corresponding author: Zalilawati Mat Rashid

Faculty of Bioresources and Food Industry,

Universiti Sultan Zainal Abidin, Besut Campus,

22200 Besut, Terengganu, Malaysia

Email: zalilawati@unisza.edu.my

Keywords:

Ziziphus mauritiana

Biochemical composition

α -Glucosidase inhibition

FTIR analysis

Polysaccharide

ABSTRACT

Ziziphus mauritiana is locally known as 'bidara' and commonly used as traditional medicine for various ailments and disease treatments. The aim of this study is to evaluate the biochemical composition and α -glucosidase inhibition of *Z. mauritiana* young leaves extracts. The fresh leaves were used to estimate the proximate values. To select young leaves, only the newly-expanded leaves at the end of small branch of plant were sampled. The leaves were extracted separately with aqueous and ethanol using ultrasonication-assisted technique, while crude polysaccharide was yielded from aqueous extract after ethanol precipitation. The ethanol (EE), aqueous (AE) and polysaccharide (PS) extracts were studied for total phenolics content (TPC), total sugars content (TSC), α -glucosidase inhibition and chemical fingerprints using Fourier transform infrared spectroscopy (FTIR). Proximate nutritional analysis revealed the presence of high moisture (70.89%) as well as crude proteins (6.92%), crude fibres (6.17%), ash (3.50%), fats (0.46%) and carbohydrates (12.0%). EE contained the most abundance TPC (24.80 mg GAE/g of extract) compared to AE (20.71 mg GAE/g of extract) and PS (3.28 mg GAE/g of extract). Meanwhile, all extracts contained high amount of carbohydrates with values 127.1 to 186.6 μ g/mL. AE and PS showed stronger α -glucosidase inhibition compared to EA with IC₅₀ values 0.959, 0.962 and 2.152 mg/mL, respectively. Thus, this research could contribute towards providing valuable scientific information to the local communities and for promoting further research on *Z. mauritiana* young leaves in Malaysia.

Keywords: *Ziziphus mauritiana*, α -glucosidase inhibition, biochemicals composition, FTIR

ABSTRAK

Ziziphus mauritiana dikenali sebagai bidara oleh masyarakat tempatan dan biasa digunakan dalam perubatan tradisional untuk mengubati pelbagai penyakit. Tujuan kajian ini adalah untuk menilai komposisi biokimia dan perencatan α -glucosidase oleh ekstrak daun muda *Z. mauritiana*. Daun yang segar digunakan untuk menganggar nilai proksimat. Hanya daun yang baru berkembang pada bahagian paling hujung ranting pokok dipilih sebagai daun muda. Daun tersebut diekstrak dengan air dan etanol menggunakan teknik bantuan ultrasonikasi manakala polisakarida mentah dihasilkan daripada ekstrak air selepas presipitasi etanol. Ekstrak etanol (EE), air (AE) dan polisakarida (PS) digunakan untuk mengkaji jumlah kandungan fenolik (TPC), jumlah kandungan gula (TSC), perencatan α -glucosidase dan pemprofilan kimia menggunakan infrared spektroskopi (FTIR). Nilai nutrisi proksimat menunjukkan kehadiran kandungan kelembapan yang tinggi (70.89%) disamping protein (6.92%), serat mentah (6.17%), mineral (3.50%), lemak (0.46%), dan karbohidrat (12.0%). EE mengandungi jumlah fenolik tertinggi (24.80 mg GAE/g ekstrak) berbanding AE (20.71 mg GAE/g ekstrak) dan PS (3.28 mg GAE/g ekstrak). Sementara itu, semua ekstrak menunjukkan kandungan karbohidrat yang tinggi dengan nilai antara 127.1 ke 186.6 μ g/mL. AE dan PS menunjukkan perencatan α -glucosidase yang lebih kuat berbanding EE dengan nilai IC₅₀ masing-masing 0.959, 0.962 dan 2.152 mg/mL. Oleh itu, kajian ini boleh menyumbang ke arah memberi informasi saintifik yang berguna kepada komuniti tempatan dan bagi menggalakkan kajian lanjutan terhadap pohon bidara di Malaysia.

Kata Kunci: *Ziziphus mauritiana*, perencatan α -glucosidase, komposisi biokimia, FTIR

INTRODUCTION

Ziziphus mauritiana belongs to family Rhamnaceae and grows in tropical and sub-tropical regions of Asia and America. It is known in Malaysia as 'bidara' and also known as Chinese apple, jujube, Indian plum or masau. Originally native to India, the plant can be found in the tropical and subtropical regions of the world. It has a wide range of morphologies from shrubs to medium sized tree with a spreading crown, stipular spines and many drooping branches. The leaves are shiny green in color, hairless above and due to persistent dense hairs, the lower surface is whitish (Mahajan & Chopda, 2009, Orwa et al., 2009). Generally, various parts of this plant are widely utilized in the traditional medicine to treat various ailments such as asthma, allergies, depression, nausea, abdominal pain in pregnancy, diabetes, ulcers and inflammation (Morton, 1987; Marwat et al., 2009; Goyal et al.,

2012). The fruits and seeds especially have been used as wound healer, anodyne, sedative, mild laxative, to halt nausea, vomiting, for pulmonary ailments and abdominal pains in pregnancy (Morton, 1987). The leaves have been used to treat asthma, fever, liver trouble, as astringent and anti-typhoid, while the powdered root is applied on wounds (Dahiru et al., 2006; Najafi, 2013). Previous scientific reports indicated the bioactivity of extracts from different parts of *Z. mauritiana* including antidiabetes (Cisse et al., 2000;), anti-inflammation (Siddharth et al., 2010), antidiarrhoeal (Dahiru et al., 2006), hypoglycemic, antioxidant (Ndhalala et al., 2006; Bhatia & Mishra, 2009; Bhatia & Mishra, 2010; Ashraf et al., 2015), antimicrobial and antitumor (Vahedi et al., 2008; Mishra et al., 2011; Ashraf et al., 2015) activities. These activities are proven to be related to its phytochemicals such as the phenolics and seed oil in fruits exhibited potential antioxidant capacity (Memon et al., 2012). Besides, the plant bark's extracts of different polarity contains phytochemicals including glycosides, alkaloids, flavonoids, glycosides, phenols, lignin, sterols, saponins and tannins can be utilized in the treatment of cancer, allergy, diabetic as well strengthening innate immune system (Jain et al., 2012).

Diabetes is one of the world's greatest health problems, affecting millions of people and most of the people were suffering from type 2 diabetes. Alpha-glucosidase inhibitors are a class of oral medications for type 2 diabetes that decrease the absorption of carbohydrates from the intestine achieving better glycemic control (Kumar, 2011). Before the absorption of carbohydrates (sugar complex form) by the intestine, they must be broken down into smaller sugar particles by natural enzymes such as alpha glucosidase. The synthetic alpha-glucosidase inhibitors include mainly acarbose, which helps in decreasing the absorption of carbohydrates from the digestive tract (Benalla et al., 2010). Presently, acarbose is an important carbohydrate metabolic enzyme inhibitor in the gastrointestinal tract, but it has some side effects such as diarrhea and intestinal disturbances. Therefore, natural plant originated inhibitors gain much more attention (Pramod et al., 2017).

Asian people also used various parts of this plant for food and medicine since decades ago. To the best of our knowledge, the few studies on this plant were carried out on antioxidant activity and phenolics content of leaves, fruits and bark (Khairul et al., 2009; Perumal et al., 2012) without focussing on the young leaves part. In this study, only young leaves of *Z. mauritiana* were being investigated as less studies have been carried out even though the young leaves are traditionally eaten as edible vegetable especially in Indonesia (Azam et al., 2006). Thus, proper documentation and scientific validation about *Z. mauritiana* potential as medicinal plant need to be conducted. The aims of the study were to identify the biochemical composition and α -glucosidase inhibition of *Z. mauritiana* young leaves extracts.

MATERIAL AND METHODS

Sample Collection and Preparation

The young and healthy leaves of *Ziziphus mauritiana* were collected in Besut, Terengganu during a sunny day. For collection of young leaves, only the newly expanded leaves at the end of small branch of the plant were sampled. Dirt was removed from the leaves. The fresh leaves were analyzed for proximate analysis and the remaining leaves stored in the freezer at -20 °C. Afterwards, the leaves samples were lyophilized, ground and weighted.

Organics and Polysaccharides Extraction

Powdered leaves (5 g) were macerated in of ethanol (25 mL). The mixture was incubated at 70 °C for 15 minutes and sonicated for 10 min. The extract was centrifuged at 4000 rpm for 15 minutes (min). Similar procedures were carried out to obtain aqueous extract (AE). Ethanol extract (EE) were concentrated under *vacuo* using rotary evaporator at 40 °C, while the aqueous extract was kept at -20 °C before lyophilization.

Isolation of crude polysaccharide was performed according to Yang (2015). Powdered leaves (5 g) were extracted with 75 mL of distilled water at 90 °C for one hours and stirred regularly. The mixture was then centrifuged and the supernatant were concentrated to 10 mL using rotary evaporator at 40 °C. Later, the fraction were precipitated drop-wise with 95% of cold ethanol (1:4, v/v). The resulting precipitate was collected by centrifugation at 4500 rpm for 15 min and later lyophilized to obtain crude polysaccharide (PS). The extracts were stored at -20 °C before used for phytochemicals and bioactivity analysis.

Proximate Composition Analysis

The young fresh leaves were analyzed for proximate composition such as crude fibre, fat, moisture content, protein, and ash content according to AOAC (2005) standard methods.

Total Phenolics Content

The amount of total phenolics content (TPC) in *Ziziphus mauritiana* leaves was determined by Folin-Ciocalteu reagent adopted from Singleton and Rossi (1965) with minor modification by Iqbal et al. (2005). 20 μ L of standard gallic acid or extract, 40 μ L distilled water (dH₂O) and 10 μ L of Folin-Ciocalteu reagent were added into 96-well plate. The mixture was mixed vigorously for 3 min followed by addition of 160 μ L of 7.5% sodium carbonate solution. The final mixture was diluted to 7 mL with dH₂O and incubate for 2 hours (h) at room temperature (RT) under dark condition. The absorbances were read at wavelength, λ 700 nm using microplate reader. The results were expressed in gallic acid equivalents (GAE) using units of mg GAE/g extract. The concentration of phenolics in the extracts were determined by plotting the absorbance value on a series of gallic acid calibration curve equation.

Total Sugars Content

Total sugars content (TSC) was determined using phenol-sulfuric acid method by Dubios et al. (1956) with slight modification. D-glucose was used as a standard to establish a calibration curve. 160 μ L of extract or D-glucose, 450 μ L of sulfuric acid (H₂SO₄) were mixed and incubated for 20 min at RT. The absorbances were read at λ 490 nm using microplate reader. The concentration of neutral sugars in the extracts were determined by plotting the absorbance values on D-glucose calibration curve equation.

α -Glucosidase Inhibition Assay

The alpha glucosidase inhibitory activity was performed according to Mayur et al. (2010) with slight modification using *p*-nitrophenyl-D-glucopyranoside (pNPG) as substrate. The substrate hydrolyzed by α -glucosidase would release *p*-nitrophenol. 10 μ L of sample was mixed with 25 μ L of α -glucosidase in 0.1 M phosphate buffer (pH = 7.0) in 96-well plate. Then, 50 μ L of 0.1 M phosphate buffer (pH = 7.0) was added. After incubating at 37 °C for 10 min, 25 μ L pNPG in 0.1 M phosphate buffer (pH = 7.0) was added. The reaction was stopped by adding 200 μ L of 0.2 M sodium carbonate (NaCO₃) and incubated at 37 °C for 30 min before the absorbance was read at λ 410 nm using microplate reader. Control sample consisted of all reagents and α -glucosidase without the test sample. The α -glucosidase inhibitory activity was expressed as percentage inhibition calculated as formula below.

$$\text{Inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where,

A₀ = absorbance of the control reaction

A₁ = absorbance of the sample

Fourier Transform Infrared (FTIR) Spectrophotometer

Spectroscopic analysis by FTIR was used to identify the characteristics of chemicals functional groups present in the extracts. Extract was evenly spread onto the crystal surface using tweezers or spatula. The pressure clamp was swing assembled so that the tip metal located above extract. The clamp was screwed down until the extract firmly sandwiched with the tip and opposing base. The analysis was carried out according to the equipment's software.

Statistical analysis

All data collected were analyzed using descriptive analysis in SPSS software version 20.0. Data was measured in triplicate and expressed as mean value $\pm$ standard deviation (SD)/standard error (SE). A one way analysis of variance (ANOVA) test was performed to investigate significant differences between the extracts and Student's *t*-test was performed to compare differences between experimental group and standard used. Data was considered significant at *p*<0.05.

RESULTS AND DISCUSSION

Proximate Composition and Percentages of Yield

In this study, the proximate composition of fresh young leaves of *Ziziphus mauritiana* collected in Besut, Terengganu is shown in Table 1. The leaves contain 70.9% moisture content, 12.1% carbohydrate, 6.9% crude protein, 6.2% crude fibre, 3.50% ash and fat content 0.46%. Moisture content was found to be higher compared

to previous work at 40.38% (Atiya *et al.*, 2011). Our study corresponds to a report revealing a common patent of the fresh young leaves of 28 Thai plants having 80% and above moisture (Maisuthisakul *et al.*, 2008). The ash, protein, crude fiber, fat and carbohydrate contents of the leaves were also found to be lower than the previously reported data (Atiya *et al.*, 2011; Akinsola *et al.*, 2016) which could possibly due to the difference in leaves maturity that effect the accumulation of biochemicals.

Table 1 Proximate composition of *Ziziphus mauritiana* fresh young leaves.

Proximate Analysis	Composition (%)
Moisture content	70.9 ± 0.1
Ash content	3.5 ± 1.8
Fat content	0.5 ± 0.1
Crude protein	6.9 ± 0.6
Crude fibre	6.2 ± 0.4
Carbohydrate	12.1 ± 0.1

*Values are mean ± standard deviation (SD) of triplicate determinations.

Table 2 shows the percentages of extracts yields obtained. The percent (%) yield of extract was calculated as the weight of the crude extract (g, W2) per dry weight of sample (g, W1). Percentage of yield of ethanolic extract (EE) obtained was the highest (24%) compared to aqueous (AE; 1.4%) and polysaccharide (PS; 1.2%). According to Do *et al.* (2014), many factors such as extracting solvent of different polarity, pH, temperature and extraction time could affect the percent yield of the extract obtained. EE appears to be dark green in color, while AE and PS are light yellow in color.

Determination of Total Phenolics Contents

In this study, total phenolics content (TPC) of three different leaf extracts ethanolic, aqueous and polysaccharide were evaluated using the Folin Ciocalteu's method. The samples TPC values were calculated from the calibration linear regression, $y = 0.016x - 0.0981$ with correlation coefficient, $R = 0.9982$ where x is the concentration of gallic acid solution (mg/mL) and y is the absorbance at λ 700 nm. The values were expressed in terms of gallic acid equivalent (mg of GAE/g of extract). The highest total phenolics content was determined in EE, followed by AE, while PS contained the least with amount of 24.80, 20.71 and 3.28 mg GAE/g extract, respectively. TPC of EE and AE were found to be insignificantly difference between one another but both were significantly different compared to PS ($p < 0.05$) (Table 2).

The result obtained were in accordance with previous investigation on *Ziziphus spina-cristi* leaves (Khaleel *et al.*, 2016) and *Ziziphus mucronata* stem revealing that ethanol was a more efficient solvent in recovering phenolics, followed by aqueous (Olajuyigbe & Anthony, 2011). Meanwhile, the presence of phenolics substituents were indeed could be derived from cell wall carbohydrate complexes as previously reported (Lygin *et al.*, 2011). Such variation in phenolics content might be linked to the chemical nature of the extracting solvent, solubility and availability of the compounds extracted (Ashraf *et al.*, 2015).

Determination of Total Sugars Content

The concentration of neutral sugars in the extracts were determined by plotting the absorbance value on D-glucose calibration curve equation, $y = 0.0082x + 0.4273$, with correlation coefficient, $R = 0.9816$. EE, AE and PS extracts showed total sugars content ranging from 127.07 μ g/mL to 186.56 μ g/mL. There were significant difference ($p < 0.05$) between TSC of extracts. AE contained 186.56 μ g/mL of TSC, followed by EE with 175.85 μ g/mL, while PS the least amount with 127.0725 μ g/mL (Table 2). Previous study stated that water is a more effective solvent for sugar extraction (Karkacier *et al.*, 2003), which correspond to the current study. Plant extracts contain diverse mixtures of sugars. The presence of glucose, fructose, galactose, sucrose, maltose,

melibiose, raffinose and stachyose have been reported from tissues of beech plant (Pak et al., 2004). Generally, the water-soluble sugars are extracted from the plant cell walls. The most common simple carbohydrates (monosaccharides) that appear as polysaccharides backbone in plant cell walls are arabinose, fucose, galactose, galacturonic acid, glucose, glucuronic acid, mannose, rhamnose, and xylose (Fang et al., 2015).

Table 2 The percentages of yields, total phenolics and neutrals sugars contents of *Ziziphus mauritiana* leaves extracts.

Extracts	Yield (%)	Total Phenolics Content (mg GAE/g extract)	Total Sugars Content ($\square$ g/mL)
Aqueous (AE)	1.40	20.71 $\pm$ 3.14 ^a	186.56 $\pm$ 2.20 ^a
Ethanol (EE)	24.00	24.80 $\pm$ 0.40 ^a	175.85 $\pm$ 3.40 ^b
Polysaccharide (PS)	1.20	3.28 $\pm$ 1.59 ^b	127.07 $\pm$ 6.60 ^c

*Values are mean $\pm$ standard deviation (SD) of triplicate determinations. Different superscript (a, b) in the same column indicates significant difference ($p < 0.05$).

α -Glucosidase Inhibition Activity

The inhibition of α -glucosidase activity by *Z. mauritiana* young leaves extracts were found to be dose dependent at concentration in range of 0.08 to 5.00 mg/mL (Figure 1). AE exhibited the strongest inhibition (92.2%), followed by SAc (88.2%), PS (85.3%) and EE (78.7%) at concentration 5.0 mg/mL. However, only the percentage of inhibition by EE was significantly different compared to standard acarbose. The concentration of extracts used to inhibit 50% of α -glucosidase activity (IC_{50}) were calculated to discuss the extracts treatment effectiveness. The lower the value, the higher the extract potential of treatment (Hamza et al., 2015). IC_{50} values of extracts were found to in range of 0.96 to 2.15 mg/mL. AE and PS showed stronger inhibitory activity with IC_{50} at 0.959 and 0.962 mg/mL, respectively, compared to EE with IC_{50} at 2.15 mg/mL. There were significant difference between IC_{50} of all extracts and standard acarbose ($p < 0.05$) (Table 3).

The presence of phenolics in both AE and EE might attribute to the potential enzyme inhibition activity of extracts. The inhibition activity differences could be expected as some bioactive components of medicinal plant may differ in their solubility and availability depending upon the extractive solvents used (Pramod et al., 2017). Previous data also stated that ethanolic extract from *Ziziphus spina-cristi* exhibited weaker inhibitory activity compared to acarbose (Hamza et al., 2015). Interestingly, there were also previous reports regarding the polysaccharides capability to inhibit α -glucosidase activity such observed in this study. For example, $\square$ -glucosidase inhibition by polysaccharides from leaves and flowers of *Camellia sinensis* (Wang et al., 2010) and polysaccharides from fruit hull of *Camellia oleifera* Abel. (Zhang & Li, 2015).

Fourier-transform Infrared Spectroscopy (FTIR) Analysis

Z. mauritiana extracts were analyzed by FTIR spectroscopy to investigate their structural diversity relatively due to extractants used and the frequencies in range of 4000 to 600 cm^{-1} are shown in Figure 2. The fingerprint region was focused between 770 and 1750 cm^{-1} in which most of chemical variability were observed (Baciu et al., 2013; Tenmerk et al., 2017). All samples contain chemical fingerprints in Area 1 (3000-3600 cm^{-1}) which indicates the characteristic of stretching vibrations of N-H bonds of proteins or hydroxyl group (O-H bonds) in water molecules, alcohols, phenolics, carbohydrates, peroxides compounds in cellulose, hemicelluloses and lignin. Area 2 (2800-3000 cm^{-1}) corresponds to stretching vibrations of the aliphatic C-H bonds in $-CH_3$ and $-CH_2$ groups in lipids and methoxy derivatives, were determined only in EE (2922.16 and 2848.86 cm^{-1}) and very small peaks in AE and PS, thus revealing EE to contain lipophilic compounds. Area 3 (1550-1750 cm^{-1}) is assigned to N-H bending and C-N stretching vibrations of amino acids, C=O stretching of aldehydes, ketones and esters, to carboxyl groups of free acids or esters (Baciu et al., 2013; Arockia, 2015) as well to C=C stretching associated

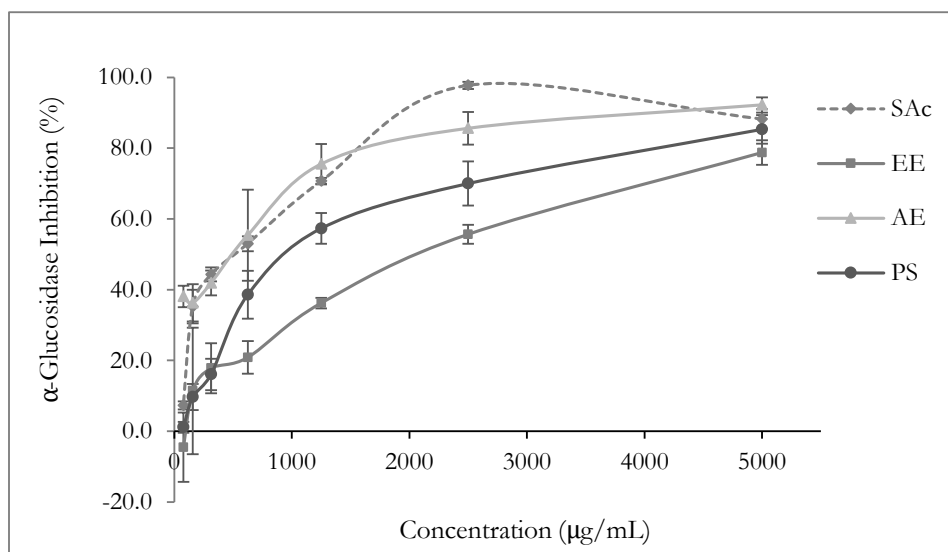


Figure 1 α -Glucosidase inhibition activity (%) of various extracts of *Ziziphus mauritiana* young leaves and standard acarbose at concentration in range of 0.08 to 5.00 mg/mL. Values are mean $\pm$ standard error (SE) of triplicate determinations. SAc: Standard acarbose; EE: ethanolic extract; AE: Aqueous extract; PS: Crude polysaccharide.

Table 3 α -Glucosidase inhibition activity (%) and IC_{50} values of standard acarbose (SAc), ethanol (EE) and aqueous (AE) extracts as well as crude polysaccharide (PS) of *Ziziphus mauritiana* young leaves.

Extracts	Mean (%) $\pm$ SEM	IC_{50} (mg/mL)
Acarbose (SAc)	88.2 $\pm$ 2.7	0.526 $\pm$ 0.05
Aqueous extract (AE)	92.2 $\pm$ 2.2	0.959 $\pm$ 0.15*
Ethanolic Extract (EE)	78.7 $\pm$ 3.4*	2.152 $\pm$ 0.13**
Crude Polysaccharide (PS)	85.3 $\pm$ 4.0	0.962 $\pm$ 0.17*

* $\square$ -Glucosidase inhibition activity (%) at sample concentration 5 mg/mL. Student's *t*-test against SAc: * $p < 0.05$, ** $p < 0.01$. Values are mean $\pm$ standard error (SE) of triplicate determinations.

with the aromatic skeletal (Pramila, 2012). Thus, EE were suggested to contains free acids (1616.35 cm^{-1}) and esters (1732 cm^{-1}), while AE contains aromatic (1589.34 and 1571.99 cm^{-1}) constituents. Area 4 (1290-1450 cm^{-1}) corresponds to C-O and C-N stretching and N-N bending of amide, C-C stretching of phenyl groups and O-H bending of alcoholic group vibrations. The spectra suggested that EE contains aromatic (1454.32 cm^{-1}) and hydroxy group (1361.74 cm^{-1}) indicates the presence of, while AE and PS also contains hydroxy group (1361.74 and 1392.61 cm^{-1}) of phenol or tertiary alcohol. Meanwhile, PS band at 1299.52 cm^{-1} showed C-N stretching assigned to aromatic primary amine compound. Besides, all extracts were found to have strong bands in Area 5 (990-1100 cm^{-1}), which corresponds to C-O stretching vibrations of glucoside bonds of carbohydrate molecule (Rashid et al., 2009). Lastly, AE and PS strong bands at 775.38 and 796.60 cm^{-1} in Area 6, respectively indicates C=C and C-C compounds (Pramila, 2012). The results of *Z. mauritiana* young leaves FTIR analysis confirmed the presence of amide, alcohols, phenols, alkanes, aldehydes, ketones, alkenes, primary amines, alkyl and aromatics skeletal, vinyl ether and alky aryl ether chemicals groups.

Comparing the fingerprints, we observed differences between the three extracts in area 2, 3, 4 and 6. EE had high abundance of area 2 suggesting that it contains higher concentration of lipophilic compounds. Besides, AE and PS were consisted of carbohydrate complex due to the sugars vibrations in frequencies domain 1400–900 cm^{-1} (Rashid et al., 2009) since they have higher intensities of peaks in Area 4 and 5 compared to EE.

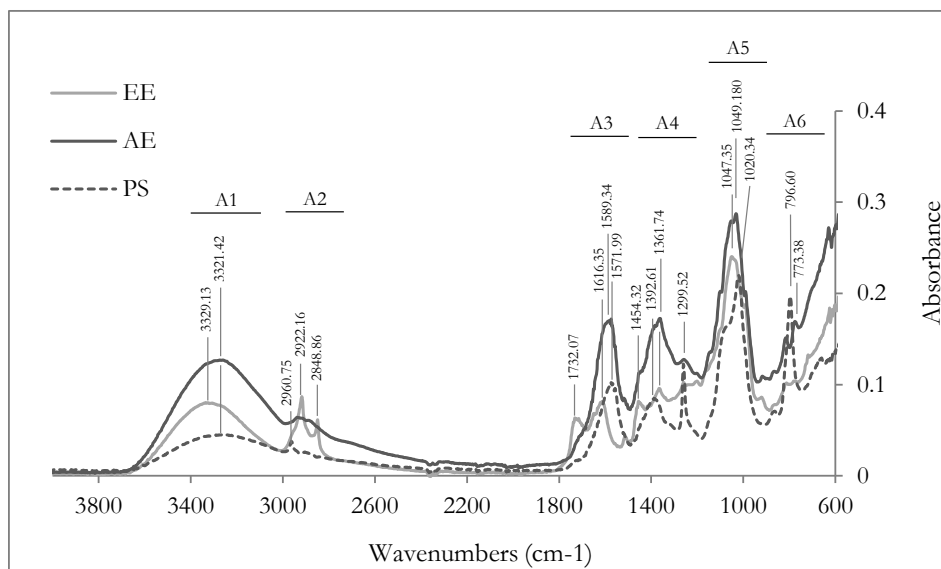


Figure 2 Fourier transform infrared spectroscopy (FTIR) fingerprints of various extracts of *Ziziphus mauritiana* young leaves. EE: ethanol extract; AE: Aqueous extract; PS: Crude polysaccharide; A1-A6: Area 1 to Area 6.

Table 3 FTIR analysis for chemical functional groups of *Ziziphus mauritiana* leaves extracts.

Fingerprints (cm^{-1})	Biochemical components
Area 1 (3000-3330 cm^{-1})	N-H stretching (proteins) or O-H stretching vibrations (water molecules, alcohols, phenolics, carbohydrates, peroxides) vibrations
Area 2 (2800-3000 cm^{-1})	C-H stretching vibrations (aliphatic bonds in $-\text{CH}_3$ and $-\text{CH}_2$ groups)
Area 3 (1550-1750 cm^{-1})	N-H bending, C-N stretching (amino acids), C=O stretching (aldehydes, ketones, esters and carbonyl), C=C stretching (aromatic skeletal) vibrations
Area 4 (1290-1450 cm^{-1})	C-O and C-N stretching and N-N bending (amide), C-C stretching (phenyl groups), O-H bending (alcoholic group) vibrations
Area 5 (990-1100 cm^{-1})	C-O stretching vibrations (glucoside bonds)
Area 6 (770-800 cm^{-1})	C=C and C-C bending vibrations

CONCLUSION

In this study, the ethanolic extract yields the highest total phenolics content, while total sugars content in aqueous extracts was most abundant compared to other extracts. The aqueous extract and polysaccharide exhibited more effective inhibition of α -glucosidase, compared to ethanolic extract. Thus, this study provided valuable information on biochemicals and potential α -glucosidase inhibition to the local communities and thus further promoting researches on Malaysian *Z. mauritiana*.

ACKNOWLEDGEMENTS

This project is funded under University Research Grant (UniSZA/2016/DPU/05), Universiti Sultan Zainal Abidin (UniSZA). The authors also thank all science officers at Faculty of Bioresources and Food Industry (FBIM), UniSZA for assistance during working with the laboratory facility.

REFERENCES

- Akinsola, R. O., Abubakar, E. I. & Zainab, A. N. (2016). Proximate and phytochemical analysis of *Ziziphus mauritiana* Lam leaves. *Journal of Biochemical Science* **1**(2): 45–49.
- AOAC. (2005). *Official Method of Analysis* (18th eds.). Association of Official Analytical Chemists International, Maryland, USA.
- Ashraf, A., Sarfraz, R. A., Anwar, F., Shahid, S. A., & Alkharfy, K. M. (2015). Chemical composition and biological activities of leaves of *Ziziphus mauritiana* L. native to Pakistan. *Pakistan Journal of Botany* **47**(1): 367–376.
- Atiya, A., Shakira, G., Asma, L. & Mukhtar, A. N. (2011). Nutritional evaluation of some top fodder tree leaves and shrubs pf district Chakwal, Pakistan in relation to ruminants requirements. *Pakistan Journal of Nutrition* **10**(1): 54–59.
- Arockia, P. S., Gowri J., Dharmalingama, V., Shobanaa, R. & Angelin, P. A. (2015). Phytochemical screening by FTIR spectroscopic analysis of leaf and stem extracts of *Wedelia biflora*. *International Journal of Nano Corrosion Science and Engineering* **2**(5): 322–334.
- Azam-Ali, S., Bonkougou, E., Bowe, C., DeKock, C., Godara, A & Williams, J. T. (2001). Fruits for the Future (Revised Edition) Ber and other jujubes. International Centre for Underutilised Crops, University of Southampton, Southampton, SO17 1BJ, UK.
- Baciu, A., Ranga, F., Fetea, F., Zavoi, S. & Socaciu, C. (2013). Fingerprinting food supplements and their botanical ingredients by coupled UV/Vis/FTIR spectrometry. *Bulletin UASVM Food Science and Technology* **70**(1): 8–15.
- Benalla, W., Bellahcen, S. & Bnouham, M. (2010). Antidiabetic medicinal plants as a source of alpha glucosidase inhibitors. *Current Diabetes Reviews* **6**(4): 247–254.
- Bhatia, A. & Mishra, T. (2009). Free radical scavenging and antioxidant potential of *Ziziphus mauritiana* (Lamk.) seed extract. *Journal of Complementary and Integrative Medicine* **8**: 42–46.
- Bhatia, A. & T. Mishra. (2010). Hypoglycemic activity of *Ziziphus mauritiana* aqueous ethanol seed extract in alloxan induced diabetic mice. *Pharmaceutical Biology* **48**: 604–610.
- Cisse, A., Ndiaye, A., Lopez-Sall, P., Seck, F., Faye, B. & Faye, B. (2000). Antidiabetic activity of *Ziziphus mauritiana* Lam (Rhamnaceae). *Dakar Medical* **45**: 105–107.
- Dahiru, D., Sini, J. M. & John-Africa L. (2006). Antidiarrhoeal activity of *Ziziphus mauritiana* root extract in rodent. *African Journal of Biotechnology* **5**(10): 941–945.

- Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S. & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis* **22**(3): 296–302.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**(3): 350–356.
- Fang, J., Qin, G., Ma, J. & She, Y-M. (2015). Quantification of plant cell wall monosaccharides by reversed-phase liquid chromatography with 2-aminobenzamide pre-column derivatization and a non-toxic reducing reagent 2-picoline borane. *Journal of Chromatography A* **414**: 122–128.
- Goyal, M., Sasmal, D. & Nagori, B. P. (2012). Review on ethnomedicinal uses, pharmacological activity and phytochemical constituents of *Ziziphus mauritiana* (Z. jujube Lam., non Mill). *Spatula DD* **2**(2): 107–116.
- Hamza, A. A., Ksiksi, T. S., Shamsi, O. A. A. L. & Balfagh, S. A. (2015). α -Glucosidase Inhibitory activity of common traditional medicinal plants used for diabetes mellitus. *Journal of Develop Drugs* **4**: 144.
- Jain, A., Bhatt, S. & Dhyani, S. (2012). Phytochemical screening of secondary metabolite of *Ziziphus mauritiana* Lamm. bark. *International Journal of Current Pharmaceutical Research* **4**(3): 156–159.
- Khairul, I. E. H., Khoo, H. E., Mhd, J. A. M., Ismail, A., Idris, S., Azlan, A., Mohd, N. H. S., Mat, D. N. A., & Mohd, M. R. A. (2009). Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *Journal of Food Composition and Analysis* **22**: 388–393.
- Khaleel, S., Jaran, A. & Haddadin, M. (2016). Evaluation of Total Phenolic Content and Antioxidant Activity of Three Leaf Extracts of *Ziziphus spina-christi* (Sedr) Grown in Jordan. *British Journal of Medicine and Medical Research* **14**(6): 1–8.
- Kumar, V., Narwal, S., Kumar, S. & Prakash, O. (2011). α -glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy Reviews* **5**(9): 19–29.
- Lygin, A. V., Upton, J., Dohleman, F. G., Juvik, J., Zabolina, O. A., Widholm, J. M. & Ozovaya, V. V. (2011). Composition of cell wall phenolics and polysaccharides of the potential bioenergy crop – *Miscanthus*. *GCB Bioenergy* **3**: 333–345.
- Mahajan, R. T. & Chopda, M. Z. (2009). Phyto-pharmacology of *Ziziphus jujuba* mill – a plant review. *Journal of Pharmacology* **3**(6): 320–329.
- Maisuthisakul, P., Pasuk, S. & Ritthiruangdej, P. (2008). Relationship between antioxidant properties and chemical composition of some Thai plants. *Journal of Food Composition and Analysis* **21**(3): 229–240.
- Marwat, S. K., Khan, M. A., Rehman, F.U., Ahmad, M., Zafar, M. & Sultana, S. (2009). *Salvadora persica*, *Tamarix aphylla* and *Zizyphus mauritiana*-three woody plant species mentioned in Holy Quran and Ahadith and their ethnobotanical uses in north western part (D.I. Khan) of Pakistan. *Pakistan Journal of Nutrition* **8**: 542–547.
- Mayur, B., Sandesh, S., Shruti, S. & Sung-Yum, S. (2010). Antioxidant and α -glucosidase inhibitory properties of *Carpesium abrotanoides* L. *Journal of Medicinal Plants Research* **4**(15): 1547–1553.
- Memon, A. A., Memon, N., Luthria, D. L., Pitafi, A. A. & Bhangar, M. I. (2012). Phenolic compounds and seed oil composition of *Ziziphus mauritiana* L. fruit. *Polish Journal of Food and Nutrition Sciences* **62**(1): 15–21.
- Mishra, T., Khullar, M. & Bhatia, A. (2011). Anticancer potential of aqueous ethanol seed extract of against cancer cell lines and Ehrlich Ascites Carcinoma. *Evidence-Based Complementary and Alternative Medicine Article*. **2011**: 1-11. <https://doi.org/10.1155/2011/765029>.

- Morton, J. (1987). Indian jujube. p. 272–275. In *Fruits of Warm Cimates*. Morton, J. F. and Miami, F. L. (eds.). Center for New Crops & Plant Products, Purdue University, Lafayette, Ind, USA. http://www.hort.purdue.edu/newcrop/morton/indian_jujube.html.
- Najafi, S. (2013). Phytochemical screening and antibacterial activity of leaf extract of *Ziziphus mauritiana* Lam. *International Research Journal of Applied and Basic Sciences* **4(10)**: 3274–3276.
- Ndhala, A. R., Mupure, C. H., Chitindingue, K., Benhura, M. A. N. & Muchuweti, M. (2006). Antioxidant potentials and degree of polymerization of six wild fruits. *Life Science Research Assays* **1**: 87–92.
- Olajuyigbe, O. O. & Afolayan, A. J. (2011). Phenolic content and antioxidant property of the bark extracts of *Ziziphus mucronata* Willd. subsp. *mucronata* Willd. *BMC Complementary and Alternative Medicine* **11**: 130. doi: 10.1186/1472-6882-11-130.
- Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Anthony, S. (2009). *Ziziphus mauritiana*. p. 1–6. In *Agroforestry Database: A Tree Reference and Selection Guide Version 4.0*. World Agroforestry Centre, Kenya.
- Pak, S. C. & Simon M. (2004). A method for routine measurements of total sugar and starch content in woody plant tissues. *Plant Physiology* **2(24)**: 1129–1136.
- Perumal, S., Mahmud, R., Piaru, S. P., Lee, W. C. & Ramanathan, S. (2012). Potential antiradical activity and cytotoxic assessment of *Zizyphus mauritiana* and *Syzygium polyanthum*. *International Journal of Pharmacology* **8(6)**: 535–541.
- Pramod, M. Ajay, S., Gopal, R. & Santram, L. (2017). Hypoglycemic and hypolipidemic effects of ethanolic and aqueous extracts from *Ziziphus oenoplia* (L) Mill on alloxan-induced diabetic rats. *Journal of Basic and Applied Sciences* **6(1)**: 1–9.
- Pramila, D. M. (2012). Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (*Mentha piperita*: *Lamiaceae*). *Journal of Medicinal Plants Research* **6(3)**: 331–335.
- Rashid, Z. M., Lahaye, E., Defer, D., Douzenel, P., Perrin, B., Bourgougnon, N. & Sire, O. (2009). Isolation of a sulphated polysaccharide from a recently discovered sponge species (*Celtodoryx girardae*) and determination of its anti-herpetic activity. *International Journal of Biological Macromolecules* **44**: 286–293.
- Siddharth, P., P. Kailash, V. Niraj, M. Karuna, P. Vimal, P. Bharadia & K. Pundarikakshudu. (2010). Antiulcer activity of methanolic extract of *Ziziphus mauritiana* stem bark. *International Journal of Pharmacognosy and Phytochemical Research* **2(3)**: 6–11.
- Shimada, K., Fujikawa, K., Yahara, K. & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry* **40(6)**: 945–948.
- Temerk, H., Salem, W. M., Sayed, W. F. & Hassan, F. S. (2017). Antibacterial effect of phytochemical extracts from *Ziziphus-spina christi* against some pathogenic bacteria. *Egyptian Journal of Botany* **57(3)**: 595–604.
- Vahedi, F., Najafi, F. M. & K. Bozari. (2008). Evaluation of inhibitory effect and apoptosis induction of *Zyzyphus jujube* on tumor cell lines, an in vitro preliminary study. *Cytotechnology* **56(2)**: 105–111.
- Wang, Y., Yang, Z. & Wei, X. (2010). Sugar compositions, α -glucosidase inhibitory and amylase inhibitory activities of polysaccharides from leaves and flowers of *Camellia sinensis* obtained by different extraction methods. *International Journal of Biological Macromolecules* **47(4)**: 534–539.
- Yang, H., Yin, T. & Zhang, S. (2015). Isolation, purification, and characterization of polysaccharides from wide *Morchella esculenta* (L.) pers. *International Journal of Food Properties* **18(7)**: 1385–1390.
- Zhang, S. & Li, X-Z. (2015). Inhibition of α -glucosidase by polysaccharides from the fruit hull of *Camellia oleifera* Abel. *Carbohydrate Polymers* **115**: 38–43.