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Bioactive fingerprints of aqueous extracts of *Ficus deltoidea* syconia via FTIR spectroscopy coupled with chemometrics

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Ficus deltoidea is a medicinal plant containing diverse bioactive phytochemicals. However, little is known about the phytochemical variation in aqueous extract of the syconia part. This study aimed to discriminate the chemical fingerprints of aqueous extracts of seven varieties of *F. deltoidea* (var. *trengganuensis*, var. *kunstleri*, var. *angustifolia*, var. *deltoidea*, var. *bilobata*, var. *intermedia* and var. *motleyana*) and their correlation with biological activities. Fourier transform infrared spectroscopy (FTIR) coupled with chemometrics applied, includes principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA) and partial least square (PLS). OPLS-DA reveals the clusters of chemical fingerprints of the samples. Loading plots of the first two principal components (PC1 and PC2) revealed wavenumbers 1001, 1093, 1435 and 1600 cm^{-1} to be the significant fingerprints responsible for chemical variability of the varieties. The *in-vitro* study revealed that var. *kunstleri* demonstrated the strongest antioxidant and α-glucosidase inhibitory activities, followed by var. *angustifolia* and var. *bilobata*. PLS model show the three varieties separation and correlation with bioactivity compared to the other varieties. The results revealed that FTIR spectroscopy with chemo metrics tools could be used for metabolomics study of bioactive structural functions.

Keywords: alpha-glucosidase, antioxidant, chemometrics, *Ficus deltoidea*, FTIR

INTRODUCTION

Ficus deltoidea is a small tree belongs to the family of Moraceae and cultivated as a houseplant or ornamental shrub (Nasir et al., 2014). It is a native of Peninsular Malaysia which is locally known as ‘mas cotek’ because of the presence of fine spots with gold color on the surface of each leaf (Mat et al., 2012). It is called Sempit-Sempit and Agoluran by people in Sabah, Sarawak, and Kangkalibang in Africa (Bunawan et al., 2014). Its leaves are commonly being processed and consumed as tea (Mohd et al., 2016). It is used in regulating blood pressure and cholesterol level (Misbah et al., 2013). Traditionally, it is a medicinal plant which various parts are used to treat several health conditions such as diabetes, headache, hypertension, fever and to reduce the risk of getting cancer (Woon et al., 2014).

Diabetes mellitus (DM) is one of the critical metabolic disorders characterized by high blood glucose level as result of either destruction of beta cell or its inability to produce sufficient insulin. Thus, the onset of diabetes may be due to lack of insulin, its action or both. The rise in blood sugar

level upon postprandial is associated with enzymes such as α-glucosidase which involve in the breaking of carbohydrates. Searching for α-glucosidase inhibitors from traditional herbal medicine to treat DM is of great interest (Zhou et al., 2014).

Antioxidants could also act as antidiabetic, anti-ulcer, anti-inflammation, as well as antimicrobial (Olayinka et al. 2012). They are compounds that protect cells from oxidative damage due to free radicals. Free radicals are known to contribute to the damages of macromolecules such as DNA, proteins, lipids and carbohydrates. Free radicals are formed as by-products (Oboh et al., 2012) during oxidative metabolism.

Metabolomics is the field of study dealing with biochemical composition of living organisms (Farag et al., 2013). This discipline assists researchers in detection of numerous biochemical compounds simultaneously and to compare samples reliably for differences and similarities (Farag et al., 2012) as well as the relationship between the chemical and biological activity (Abdul-Hamid et al., 2016). One of analytical tools used in metabolomics is Fourier transform infra-red (FTIR) spectroscopy. FTIR spectroscopy is a fast, non-destructive and sensitive technique, which has been widely used for chemicals fingerprinting.

Previously, the bioactive fingerprints in the ethanolic extracts of syconia of seven varieties of *Ficus deltoidea* using FTIR spectroscopy coupled with chemometrics has been evaluated (Yunusa et al. 2018). Although ethanol and water are the most preferred, there is a need to characterize the bioactive fingerprints in the aqueous extracts. Hence, this work aimed to discriminate the aqueous extracts of syconia of seven varieties of *F. deltoidea* and to correlate these varieties with their antioxidant and α-glucosidase inhibitory activities using FTIR-based metabolomics coupled with chemometrics. The findings will serve the purpose of identifying the most promising variety to be used as a functional food ingredient.

MATERIALS AND METHODS

Chemicals and reagents

2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium acetate trihydrate and ferric chloride were purchased from Sigma-Aldrich Co. (Switzerland). Acetic acid was bought from R & M chemicals. Hydrochloric acid was purchased from Merck (Germany). α-glucosidase enzyme and *p*-nitrophenyl-α-D-glucopyranose (PNPG) were supplied by Sigma-Aldrich (St. Louis, MO, USA). All other solvents and chemicals used were of analytical grade.

Sample collection, preparation and extraction

Syconia of seven varieties of *Ficus deltoidea* were collected from the germplasm of Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin. All syconia were carefully selected to be identical in terms of color and ripening stage. The syconia of the seven varieties were deposited as voucher specimens at the faculty's herbarium. The syconia samples were dried and then ground into powder form and stored at –20 ºC before extraction.

Dried and powdered syconia were extracted with water in a Soxhlet apparatus. Approximately, 20 g of ground sample was weighed into a thimble and was extracted with 200 mL (1:10 (w/v)) of water for 5 hr. The extract was filtered and concentrated under reduce pressure (72 mbar) at 40 °C using rotary evaporator and completely dried using freeze drier. Then the crude extracts were stored at -20 °C until analysis.

Bioactivity Analysis

DPPH radicals scavenging assay

The radical scavenging ability of samples was measured following the method of Shimada et al. (1992) and modified by Farsi et al. (2014). A volume of 200 µL of DPPH methanolic solution (0.004% w/v) was added to 100 µL of sample at different concentrations. The mixture was incubated for 30 min at room temperature in the dark. Reduction of DPPH was measured at 517 nm. The percentage of scavenging activity was evaluated by comparing with the control (a mixture of 100 µL methanol and 200 µL DPPH). Quercetin was used as a reference standard. The radical scavenging activity was calculated by using the following formula:

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

Where; A_o is the absorbance of the control reaction; A_1 is the absorbance of the sample itself.

The inhibitory concentration at 50% (IC_{50}) values (extract concentration that causes 50% scavenging of DPPH radical) was determined from the graph of scavenging percentage against the extract concentrations (conc.: 1.56, 3.13, 6.25,

12.5, 25, 50 and 100 µg/mL). All determinations were carried out in three different experiments.

Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power (FRAP) was measured according to Benzie and Strain, (1996) and modified by Thaipong et al. (2006). A working FRAP solution was prepared by mixing 300 mM acetate buffer, 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3$ in the ratio of 10:1:1, and warmed up at 37 °C for 10 min in a water bath before used. A volume of 15 µL of sample extract (100 µg/mL) was added with 285 µL of the working FRAP solution and incubated at room temperature in the dark for 30 min. The absorbance was read at 593 nm. Ferrous sulfate ($FeSO_4.7H_2O$) with concentrations between 125 to 1000 µM was used as a standard and the results were expressed as millimoles of Fe^{2+} equivalents per gram of dried extract (mmol Fe^{2+}/g).

α-Glucosidase inhibition assay

The alpha-glucosidase inhibition assay was performed according to Mohd et al. (2016), with slight modification. A volume of ten µL of samples at different concentrations (0, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 µg/mL) were mixed with 50 µL of 0.1 M phosphate buffer (pH 7.0) and 25 µL of alpha-glucosidase in phosphate buffer (pH 7.0) (0.2 U/mL), in 96-well plate. The plate was incubated for 10 min at 37 °C to initiate the reaction. A volume of 25 µL of 0.5 mM 4-nitrophenyl-α-D-glucopyranoside (PNPG) substrate was added to complete the reaction and incubated (30 min, 37 °C). The reaction was terminated by adding 100 µL of 0.2 M sodium carbonate solution. Quercetin was used as a positive control. The absorbance was measured at 410 nm. The percentage of inhibition was calculated by using the following formula:

Inhibition (%) = [(ABS Control – ABS Sample)/ABS Control] × 100

ATR-FTIR analysis

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra of extracts were gathered using Shimadzu Prestige-21 Spectrophotometer (Shimadzu, Nakagyo-ku, Kyoto, Japan) equipped with a DTGS KBr detector and a Golden Gate Single Reflection Diamond ATR accessory (incident angle of 45°). The spectra of absorbance mode at mid-infrared region (4000–400 cm^{-1}) were recorded using 16 scans and 4 cm^{-1} resolution. Each analysis was done in triplicate.

Statistical analysis

Results are presented as the mean ± standard deviation (SD) of three different experiments. Analysis of variance (ANOVA) was used to determine the differences on antioxidant and alpha-glucosidase inhibitory activities. The alpha level of all analysis was at 0.05.

Data pre-processing and multivariate analysis

Each spectrum was baseline corrected and smoothed to 7 data points (Kaya-Celiker et al. 2015) by using spectrum software to minimize the differences between spectra due to baseline shifts and then exported to ASCII file. The excel file was used for supervised and unsupervised chemometrics analysis. Supervised multivariate data analysis is used to construct a valid model that perfectly associates inputs with outputs which may be linear or nonlinear (Nicoulaou et al., 2010), The multivariate data analyses (MVDA) performed were principal component analysis (PCA), OPLS-DA and PLS. X is the wavenumber from the FTIR spectra and Y represented biological activities (DPPH, FRAP and α-glucosidase inhibition). This MVDA were performed using the SIMCA-P software (v. 13.0, Umetrics, Umeå, Sweden) with the UV scaling method.

RESULTS

Bioactivity

DPPH radicals scavenging activity

The results of DPPH radical scavenging activity (%) varied between 23.9 to 83.8%. The highest percentage was found in the accession of var. *intermedia* (I386), while var. *motleyana* (M234A) recorded the lowest scavenging activity. Based on the IC_{50} var. *kunstleri* (K003) demonstrated the highest activity (Table 1).

Ferric reducing antioxidant power (FRAP)

The results of the reducing power of syconia of seven varieties of *Ficus deltoidea* indicated that the ability of the extracts to reduce Fe^{3+} to Fe^{2+} varied from 1.48 to 6.81 mmol/g. Accession from var. *kunstleri* (K003) showed the highest reducing power value, while that of var. *motleyana* (M234A) showed the lowest reducing power (Table 1).

Table 1: DPPH radical scavenging, Ferric reducing power (FRAP) and α-glucosidase inhibitory activities of aqueous extracts (AE) of syconia of seven varieties of *Ficus deltoidea*

Accession Code	DPPH Scavenging Activity (%)	DPPH Scavenging Activity (IC_{50}; µg/mL)	FRAP (Fe^{2+}; mmol/g)	α-Glucosidase Inhibitory Activity (%)	α-Glucosidase Inhibitory Activity (IC_{50}; µg/mL)
T002	81.12 ± 1.40^{abcd}	44.67 ± 1.53^{d}	3.25 ± 1.18^{cdef}	39.04 ± 4.31^{fg}	ND
T019	63.62 ± 4.72^{e}	70.33 ± 6.64^{ab}	4.02 ± 1.46^{bc}	48.27 ± 5.40^{ef}	ND
T020	63.11 ± 3.76^{e}	75.27 ± 2.65^{a}	2.06 ± 0.13^{fg}	19.75 ± 0.23^{hij}	ND
K003	81.07 ± 10.01^{abcd}	21.00 ± 1.73^{g}	6.81 ± 0.27^{a}	77.36 ± 9.35^{ab}	57.67 ± 9.87^{e}
K217	78.93 ± 10.21^{abcd}	34.00 ± 1.00^{ef}	4.61 ± 0.30^{b}	54.77 ± 1.45^{de}	90.83 ± 2.36^{ab}
K313	80.17 ± 10.26^{abcd}	34.75 ± 4.13^{def}	4.64 ± 0.24^{b}	78.42 ± 16.04^{ab}	77.17 ± 6.83^{abcd}
A171	80.27 ± 10.98^{abcd}	29.83 ± 2.02^{efg}	4.70 ± 0.24^{b}	79.30 ± 5.93^{a}	57.58 ± 16.01^{e}
A321	81.14 ± 3.26^{abcd}	38.83 ± 3.01^{de}	3.59 ± 0.22^{bcde}	73.41 ± 13.33^{abc}	74.17 ± 3.51^{bcde}
A295	32.57 ± 2.28^{g}h	ND	2.75 ± 2.02^{defg}	16.39 ± 4.66^{ij}	ND
D006	70.50 ± 8.21^{cde}	65.00 ± 12.44^{abc}	3.74 ± 0.26^{bcd}	23.57 ± 10.68^{hij}	ND
D172	72.02 ± 0.66^{bcde}	63.33 ± 1.76^{bc}	3.56 ± 0.42^{bcde}	22.31 ± 1.42^{hij}	ND
D156	62.35 ± 3.39^{e}	74.67 ± 8.33^{a}	3.04 ± 0.17^{cdef}	38.30 ±4.91^{fg}	ND
B013	64.87 ± 5.14^{e}	73.33 ± 1.53^{ab}	1.97 ± 0.19^{fg}	29.37 ± 6.77^{ghi}	ND
B014	82.05 ± 10.03^{abc}	27.33 ± 3.51^{fg}	4.66 ± 0.29^{b}	64.75 ± 6.92^{bcd}	83.50 ± 5.68^{abc}
B378	81.25 ± 10.42^{abc}	30.83 ± 2.84^{efg}	4.19 ± 0.28^{bc}	72.42 ± 7.24^{abc}	80.67 ± 1.04^{abcd}
I323	44.57 ± 0.38^{f}	ND	2.44 ± 0.89^{efg}	74.10 ± 11.30^{abc}	81.25 ± 7.43^{abcd}
I386	83.78 ± 1.55^{ab}	35.50 ± 4.77^{def}	4.58 ± 0.07^{b}	55.26 ± 4.97^{de}	93.67 ± 4.62^{a}
I378	68.31 ± 9.78^{de}	58.33 ± 13.50^{c}	2.48 ± 0.52^{defg}	81.01 ± 6.64^{a}	71.00 ± 7.55^{cde}
M234A	23.90 ± 5.17^{h}	ND	1.48 ± 0.14^{g}	14.90 ± 5.80^{j}	ND
M234B	39.03 ± 7.03^{g}	ND	2.23 ± 0.09^{fg}	39.72 ± 7.91^{fg}	ND
M234C	47.14 ± 2.44^{f}	ND	2.06 ± 0.14^{fg}	32.32 ± 2.57^{gh}	ND
Quercetin	87.32 ± 2.26^{a}	5.50 ± 0.87^{h}	ND	63.01 ± 5.90^{cd}	65.17 ± 18.87^{de}

Values are the means ± standard deviation (n=3) at sample concentration 100 µg/mL. Superscript letter refers to significant different (p<0.05) by comparing among syconia accessions. Means with different superscript letters were significantly different (p<0.05). IC_{50}: inhibitory concentration. ND: not detected T: var. *trengganuensis*, K: var. *kunstleri*, A: var. *angustifolia*, D: var. *deltoidea*, B: var. *bilobata*, I: var. *intermedia*, M: var. *motleyana*.

Alpha-glucosidase inhibition activity

The ability of extracts of different accessions to inhibit alpha-glucosidase (%) evaluated in this study varied from 14.9 to 79.3%. Among the extracts, accessions from var. of *kunstleri* and *angustifolia* (K003 and A171) were the most efficient inhibitors of alpha-glucosidase with an IC_{50} value of 57.67 and 57.58 µg/mL, respectively (Table 1).

FTIR analysis of *Ficus deltoidea* varieties

The representative FTIR spectra of aqueous extracts of syconia of seven varieties of *Ficus deltoidea* are given in Figure 1. The spectra shows broad peaks in Area 6 and intense peaks in Area 3 and 4 fingerprint regions. The spectra in Area 1 suggest the presence of O-H bonds of either phenols, alcohols or water fraction.

The presence of macro-molecule such as lipids, proteins, polysaccharides can be deduced by the specific functional groups at wavenumbers ranging from 1800 and 800 cm^{-1} (Lu et al., 2011). Besides, peak at about 1043.5 cm^{-1} assigned for vibration of amines. Area 5 region indicates the presence of C-O stretching vibrations of glucoside bonds (García et al., 2013) (Table 2).

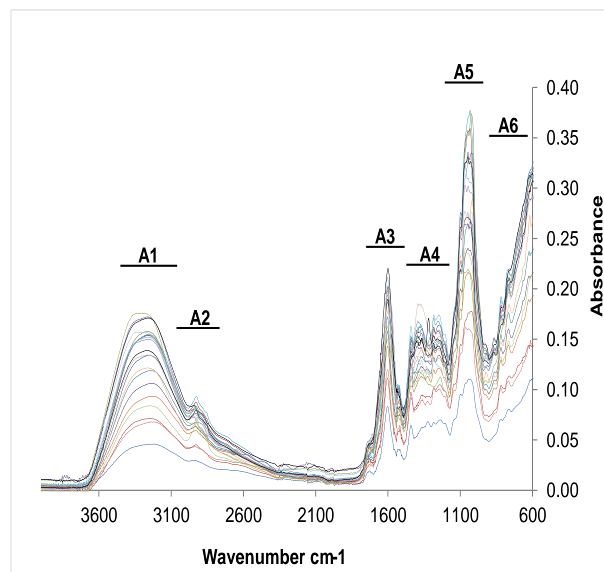

Figure 1: Overlay FTIR spectra of aqueous extracts of syconia of seven varieties of *Ficus deltoidea*
*** A1-A6; Area 1 to Area 6, T: var. *trengganuensis*, K: var. *kunstleri*, A: var. *angustifolia*, D: var. *deltoidea*, B: var. *bilobata*, I: var. *intermedia*, M: var. *motleyana*.**

Table 2; Functional group and mode of vibration in aqueous extracts of *Ficus deltoidea* varieties

Frequency Range (cm^{-1})	Functional Groups Vibrations
Area 1 (3600-3000)	O-H stretching (water molecules, alcohols, phenolics, carbohydrates, peroxides) vibrations
Area 2 (2900 – 2800)	C-H stretching vibrations (aliphatics bonds in $–CH_3$ and CH_2 groups)
Area 3 (1740-1580)	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)	C-O and C-N stretching and N-N bending (amide), C-C stretching (phenyl group), O-H bending (alcoholic group) vibrations
Area 5 (1100-990)	C-O stretching vibrations (glucoside bonds)
Area 6 (800-770)	C=C and C-C bending vibrations

FTIR-based metabolomics and chemometrics

Principal component analysis (PCA)

FTIR data sets of aqueous extracts were subjected to PCA analysis as unsupervised classification of syconia of the seven varieties of *Ficus deltoidea*. In PCA analysis, the first two PCs covered 90.1% variance with R^2X (cum) and Q^2 (cum) of 0.993 and 0.983, respectively. The presence of outliers was located outside the 95% confidence level of hotelling's T^2 of PCA (Figure 2A.). The major fingerprints for variation were 997, 460 and 466 cm^{-1} along PC1 (Figure 2B) and 1600 and 995 cm^{-1} along PC2 (Figure 2C).

Orthogonal partial least Square – discriminant analysis (OPLS-DA)

For the supervised discrimination of syconia of seven varieties of *Ficus deltoidea*, OPLS-DA model was established. The model was validated by the Q^2Y and R^2Y, OPLS-DA clearly separated and discriminated the varieties of syconia of *Ficus deltoidea* on the PC1 (Figure 3A). The model had an overall R^2Y (cum) as 0.904 and Q^2 (cum) as 0.777.

Three varieties consisting of var. *motleyana*,

var. *trengganuensis* and var. *deltoidea* were located on the right side of the plot, while var. *kunstleri*, var. *bilobata* and var. *angustifolia* were discriminated to the left side (Figure 3A) and var. *intermedia* was in the center of the plot. Meanwhile, separation of var. *deltoidea* from the other two varieties was revealed on PC2, in which the positive side of PC2 shows discrimination of var. *kunstleri* from other varieties. Loading plots of PC1 and PC2 revealed wavenumbers of 1001, 1093, 1435, 1600 and 1969 cm^{-1} to be the major fingerprints responsible for the varieties discrimination (Figures 3B & C).

OPLS-DA-HCA

OPLS-DA-HCA was generated, and relationships between varieties were calculated using Euclidean distance, and the clusters were formed using ward hierarchical agglomerative method. The HCA was presented in the form of dendrogram which showed the clustering of the aqueous extracts of seven varieties of *Ficus deltoidea* (Figure 3D). The OPLS-DA-HCA model allowed the formation of only two clusters (Figure 3D). C1 was formed by accessions of var. *deltoidea*, var. *trengganuensis* and var. *motleyana*, and C2 was formed by accessions of var. *kunstleri*, var. *angustifolia*, var. *bilobata* and var. *intermedia*.

Partial least square (PLS)

PLS model was generated to determine the relationship between the bioactivity and FTIR fingerprints of seven varieties of *Ficus deltoidea*. X was the set of wavenumbers (400-4000 cm^{-1}) and Y represented biological activities (DPPH, FRAP and α-glucosidase inhibition). The PLS model of seven varieties was cross-validated using 100 permutation tests. The Q^2 values were lower than those of original ones. The cumulative R^2X, R^2Y and Q^2 values suggested good predictive abilities ($R^2X = 0.812$; $R^2Y = 0.656$; $Q^2 = 0.589$). The bi-plot of the extracts showed that var. *kunstleri* var. *angustifolia*, and var. *bilobata* were located in the right side of PC1. On the other hand, var. *deltoidea*, var. *motleyana* and var. *trengganuesis* possessed the lowest biological activity and located on the negative side of PC1 (Figure 4).

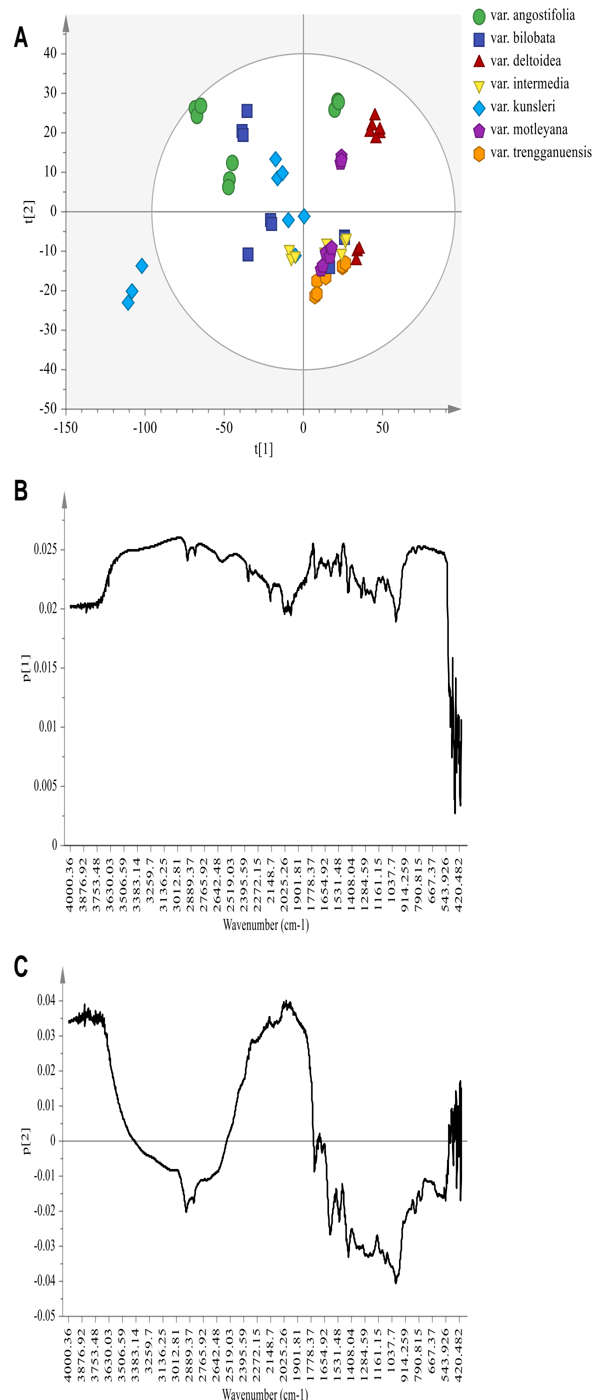

Figure 2: Unsupervised multivariate data analysis (MVDA) of ATR-FTIR spectra of aqueous extracts of syconia of seven varieties of *Ficus deltoidea*. A) Principal component analysis (PCA) score plots, B) loading line plots of PC1, and C) loading line plots of PC2.

DISCUSSION

Bioactivity of *F. deltoidea* varieties

Few studies on syconia part of some varieties of *F. deltoidea* have been found compared to the leaf part. Misbah et al., (2013), reported that the aqueous extracts of syconia of *Ficus deltoidea* var. *angustifolia* was found to possess higher activity with IC_{50} of 111.20 ± 4.77 µg/mL compared to the var. *kunstleri* which had IC_{50} of 150.25 ± 3.05 µg/mL. The radical scavenging activities of different varieties of *Ficus deltoidea* reported by Dzolin et al., (2015) are in agreement with outcomes of the present study. Previous study reported the scavenging activity of *F. deltoidea* (49.6%) (Wan Mustaffa et al., 2015), which was lower than most of the accessions used in the present study. The FRAP assay is based on the ability of the plant or food extract to reduce Fe^{3+} to Fe^{2+} in the presence of antioxidants. Previously, FRAP values of aqueous extracts of *Ficus deltoidea* var. *angustifolia* and var. *kunstleri* were respectively, 1.82 ± 0.19 and 1.27 ± 0.11 mmol/g (Misbah et al. 2013) which were significantly lower than the syconia of the similar varieties used in the present study.

In an attempt to screen the potential of syconia of *Ficus deltoidea* as an antidiabetic agent, the *in vitro* α-glucosidase inhibition assay was conducted. α-glucosidase inhibitory activity (%) of butanolic extract of *F. deltoidea* at 1 mg/mL was reported to be 55% and significantly higher than that of hexane, chloroform and residual extracts (Choo et al. 2012). Compared to the concentration used in the previous study, the present study exhibited stronger inhibition at lower concentration against α-glucosidase enzyme. The present study revealed that variety of *trengganuensis*, *deltoidea* and *motleyana* show no IC_{50} at 100 µg/mL. The α-glucosidase inhibitory activity (IC_{50}) reported by Misbah et al. (2013) were 65.00 ± 5.00 µg/mL in the aqueous extract of var. *angustifolia* while 61.00 ± 3.00 µg/mL in the extracts of var. *kunstleri*, which were consistent with our present study.

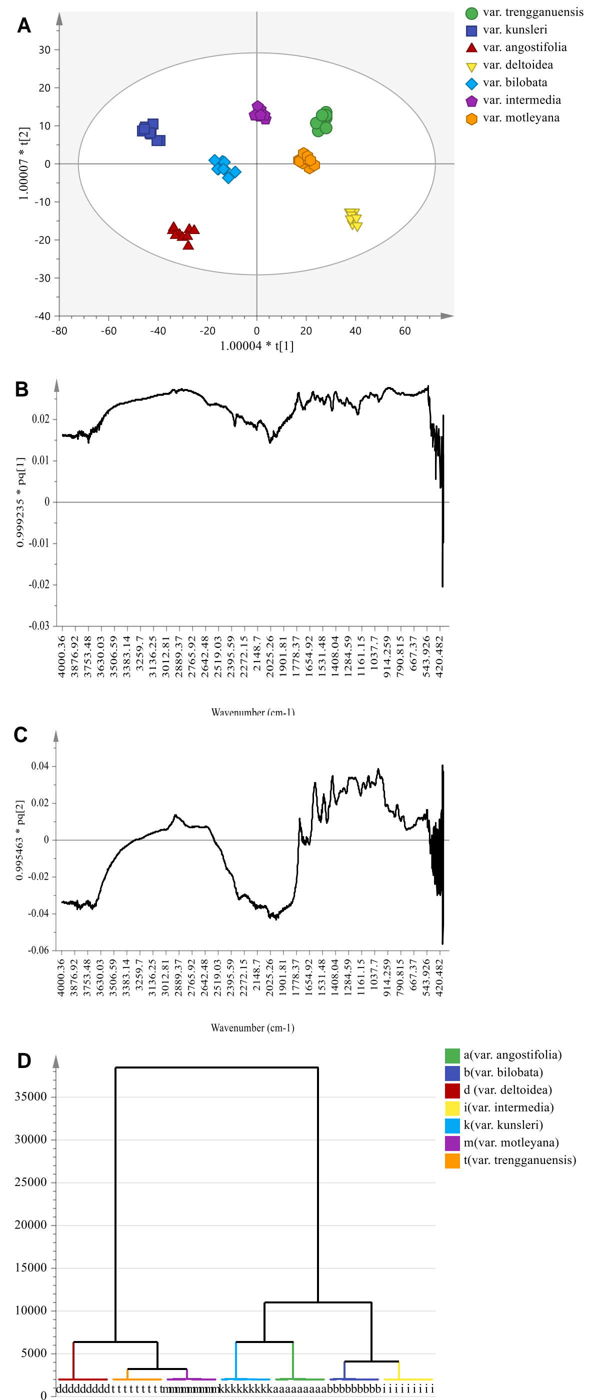

Figure 3: Supervised multivariate data analysis (MVDA) of ATR-IR spectra of aqueous extracts of syconia of seven varieties of *Ficus deltoidea*. A) Orthogonal partial least square discriminant analysis (OPLS-DA) score plots, B) OPLS-DA loading line plots of PC1, C) OPLS-DA loading line plots of PC2, and D) OPLS-DA-Hierarchical cluster analysis (OPLS-DA-HCA).

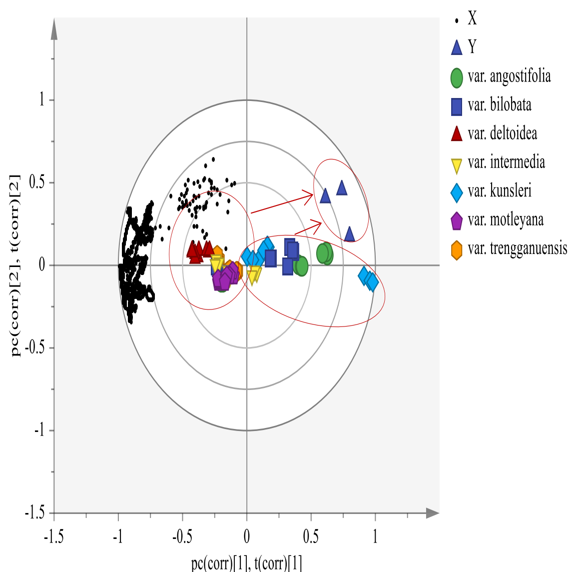

Figure 4: The PLS loading bi-plot of syconia extracts obtained from seven varieties of *Ficus deltoidea*

FTIR analysis of *Ficus deltoidea* varieties

FTIR fingerprints of aqueous extracts were found slightly similar with that of ethanolic extracts reported by Yunusa et al., (2018), though some dif-ferences in the absorbance intensities were observed. For example, peaks at 2854.65 cm^{-1}, 1732.08 to 1710.86 cm^{-1} and 1162 cm^{-1}. Easmin et al. (2016) also observed spectral differences between the aqueous and ethanolic extract of *Phaleria macrocarpa* which are attributed to the slight difference in solvating properties of water and ethanol. Besides, spectra of some accessions of (T020, D006, I323, M234A) had lower intensity peaks at 1732-1710 cm^{-1} which indicate the presence of C=O, while others (T019, A171, A295, D172, D156, B013) did not contain the functional group. In the same vein, the aqueous extracts of *Ficus deltoidea* var. *borneensis* also reported the absence of a peak at 1710-1740 cm^{-1} (Azemin et al., 2014). This finding corresponded to a published study of stronger bioactivity (α-glucosidase inhibitory activity) of ethanolic extracts of *Cosmos caudatus*, which ascribed due to the phenolic compounds found only in the ethanolic extracts as compared to the water extracts (Javadi et al., 2014).

FTIR-based metabolomics and chemometrics

Principal component analysis (PCA)

In an attempt to determine the relationships between different varieties of *F. deltoidea*, PCA model was established to find the variation in the data set and obtain an overview of the samples between groups (Kim et al., 2013). Score plot was used to find out the clusters of different samples, with the corresponding loading plots demonstrating the variables responsible for the most variation in the specified PC. In the present study, there were no defined clusters (Figure 2A). Similar to our findings, previous study also did not observe clear separation to discriminate cabbage cultivars (Kim et al., 2013), *Ipomoea aquatica* (Lawal et al., 2016) and yerba mate (Marcelo et al., 2015). The obvious reason for the overlapping was determined to be due to the similarity in their phytochemicals content. Thus, due to their distinctive metabolic characteristics, samples of green tea from Sri Lanka were clustered differently from the other origins (Fraser et al., 2013). Further, PCA established a defined classes of serrano peppers (*Capsicum annum* L.) grown from two different regions (Becerra-Martínez et al., 2017).

Orthogonal Partial Least Square – Discriminant Analy-sis (OPLS-DA)

Discriminant analysis was used to find the mathematical tool for recognising the membership to a proper class based on the FTIR data (Fadzlillah et al., 2013). The discrimination among samples is complex to understand, and it may however, ascribed to many factors such as climate, soil type, and fertilization. These factors have greater influence in the production of metabolites (Kim et al., 2013). A random permutation test of 100 was performed on the OPLS-DA model to validate the differences between the samples (Lawal et al. 2015). OPLS-DA loading plots of the two principal components were constructed in order to understand the variables (functional group) responsible for the differentiation (Figures 3A & C).

Hierarchical cluster analysis (HCA) model of OPLS-DA revealed a number of clusters that corroborated the results obtained from HCA as derived from PCA model (data not shown). However, HCA model of OPLS-DA gave defined clusters involving all accessions of each variety as compared to HCA generated from PCA which shows the overlap of accessions from same varieties (data not shown). A similar trend was also observed in the ethanolic extracts of seven varieties of *Ficus deltoidea* (Yunusa et al. 2018). The authors conclude that, supervised HCA model of OPLS-DA give better hierarchical analysis than can unsupervised HCA of PCA model.

Partial Least Square (PLS)

In our present study, PLS model consisted of two variables; the bioactivity (DPPH, FRAP and α-glucosidase inhibitory) are the Y-axis while wavenumbers are the X-axis (4000-400 cm^{-1}). PLS, a supervised MVDA was used to establish the relationships between the bioactivity and metabolites responsible for the bioactivity of aqueous extracts (Sajak et al., 2016). The relationships between the bioactivity and different varieties of *F. deltoidea* are shown in a bi-plot, a combination of score and loading plot in one diagram, to allow easy interpretation of the relationships between both variables and observations (Figure 4). PLS model showed separation of var. *kunstleri*, var. *angustifolia* and var. *bilobata* from other varieties and showed strong correlation with bioactivity. This shows that varieties with weaker bioactivity possessed some compounds with an antagonistic effect which caused the discrimination. Thus, the model reveals that these varieties contained most of the bioactive components as compared to less bioactive ones on the left side of PC1 of the score plot. These trends agreed with our results of biological activity revealing that all the three accessions of syconia from var. *kunstleri* had stronger bioactivities. Sajak et al., (2016) also reported a clear separation between extracts having strong bioactivity and that of the weaker samples. Besides, one cultivar of *Ipomoea aquatica* showed correlation to their antioxidant and α-glucosidase inhibitory activities which made the cultivar to be separated from the others with less bioactivity (Lawal et al., 2016).

In this study, fingerprint at 459 cm^{-1} that has the highest w*c[1] was found in the same direction with α-glucosidase inhibitory activity which might be responsible for the strongest bioactivity in the extracts of var. *kunstleri*, var. *bilobata*, and var. *angustifolia* (Figure 4). Easmin et al., (2016), reported that peaks with positive pq[1] are responsible for the α-glucosidase inhibitory activity in *Phaleria macrocarpa* extracts, while peaks with negative pq[1] are identified to be responsible for decelerating the α-glucosidase inhibitory activity.

CONCLUSION

ATR-FTIR based metabolomics approach has been applied to successfully study the fingerprints variation in the syconia of aqueous extracts of seven varieties of *Ficus deltoidea*. FTIR fingerprinting was coupled with both supervised and unsupervised MVDA and visualised the variations in the extracts relative to different accessions and their antioxidant and alpha-glucosidase inhibitory activities. To the best of our knowledge, this is the first study to discriminate the aqueous extracts of syconia of seven varieties of *Ficus deltoidea* and to predict the bioactivity. This work concludes that an FTIR-based metabolomics approach can be applied to *Ficus deltoidea* in order to select the best (elite) variety for further development as an ingredient in food products and health supplement.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

AMA, CAAB, NM and ZMR designed the study and supported all the materials, AKY performed the experiments, AKY and ZMR wrote the manuscript. All authors read and approved the final version.

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