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Original Article

Chemicals and Bioactivity Discrimination of Syconia of Seven Varieties of *Ficus deltoidea* Jack via ATR-IR Spectroscopic-Based Metabolomics

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ABSTRACT

Introduction: *Ficus deltoidea* is one of the common Malaysian medicinal plants and currently commercialized as raw ingredients in some local food products. However, those products do not discriminate the varieties of *Ficus deltoidea* used. **Methods:** FTIR-based metabolomics coupled with chemometric technique was applied to discriminate chemical components in ethanolic extracts of syconia of seven varieties of *Ficus deltoidea* namely; var. *deltoidea*, var. *trengganuensis*, var. *kunstleri*, var. *angustifolia,* var. *bilobata,* var. *intermedia* and var. *motleyana*. Unsupervised multivariate data analysis (MVDA) including principal component analysis (PCA) was used as to evaluate chemical variability among the seven varieties. For discrimination, orthogonal partial least square discriminant analysis (OPLS-DA) was applied, while partial least square (PLS) was used to evaluate the relationship between the alpha-glucosidase inhibition, antioxidant activity and *Ficus deltoidea* varieties. **Results:** As a result, OPLS-DA successfully discriminated the seven varieties. The FTIR fingerprints which were responsible for the discrimination includes 1729, 1705, 1448, 1095, 453, 443 cm^{-1}. In addition, PPLS model demonstrated the correlation between var. *kunstleri*, var. *deltoidea* and var. intermedia respective chemicals fingerprints and their bioactivity (DPPH, FRAP and α-glucosidase inhibition). **Conclusion:** The findings revealed that FTIR spectroscopy, in combination with MVDA, can be used for structural functional discrimination in relation to the sample bioactivity.
Key words: Alpha-glucosidase Inhibition, Antioxidant Activity, Fourier Transform Infra-red Spectroscopy, Orthogonal Partial Least Square Discriminant Analysis, Principal Component Analysis.

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INTRODUCTION

FTIR spectroscopy is a fast, non-destructive and sensitive technique, which has been widely used for chemicals fingerprinting. It is suitable for natural products bioactive compounds analysis,[1] since no two compounds will have the same spectra.[2] It has been used for comparison and discrimination of plant/food from different origins and cultivars, such as almond cultivars subjected to oxidative treatments.[3]

Ficus deltoidea belongs to the family of Moraceae. It is distributed throughout the Southeast Asia as well as Africa.[4] In Malaysia, it is called mas cotek or sempit-sempit, while in Indonesia is known as tabat barito. Its other vernacular names are Delta fig, Fig shrub and Mistletoe.[4] It is a herb with various therapeutic benefits such as for reducing diabetic symptoms and other health risk related conditions such as obesity.[5] Various studies have applied spectroscopy coupled with MVDA to examine the differences/similarity in cultivars or plant varieties.[6] Previously, the six varieties that showed leaf morphological variations by quantitative measurement on different parts of the plant, are: var. *deltoidea*, var. *angustifolia*, var. *trengganuensis*, var. *bilobata*, var. *intermedia* and var. *kunstleri*.[7]

However, to the best of our knowledge, no literature is available for the discrimination of syconia of different varieties of *F. deltoidea*. Therefore, this study was aimed to chemically discriminate the syconia of seven varieties of *F. deltoidea* and to determine the correlation with their bioactivities.

MATERIALS AND METHODS

Sample collection and preparation

Syconia of seven varieties of *Ficus deltoidea* (3 accessions from each variety) 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 colour and ripening stage. The syconia of the seven varieties were deposited as voucher specimens at the Faculty's herbarium. The syconia samples were dried

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and then ground into powder form and stored at –20ºC before extraction.

Chemical and reagents

Ferric chloride, 2, 4, 6-tris(2-pyridyl)-s-triazine, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), sodium acetate trihydrate, were purchased from Sigma-Aldrich Co. (Switzerland). Acetic acid was purchased 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 solvents used for extraction and other chemicals were of analytical grade.

Extraction

Dried and powdered syconia were extracted individually with ethanol in a soxhlet apparatus. The extraction was repeated twice after decanting off prior extracts. Each combined ethanol were filtered, pooled and concentrated under reduced pressure at 40°C to yield crude ethanolic extracts (EE). The dried extracts were stored at -20 °C before used.

Biological activities assays

DPPH radical scavenging ability of samples were measured following,[8] and modified by.[9] FRAP was measured according to,[10] and modified by.[11] The alpha glucosidase assay, was performed according to,[12] using alpha glucosidase enzymes from *Saccharomyces cerevisiae* at different concentrations (1.56 to 100 µg/mL) as substrate and quercetin as standard.

ATR-FTIR analysis

ATR-FTIR spectra of extracts were collected by using a Bruker Analitik IFS 66 FTIR spectrometer (Ettlingen, Germany) equipped with a DTGS KBr detector and a Golden Gate Single Reflection Diamond ATR accessory (incident angle of 45°). Spectra were recorded in the absorbance mode at mid infrared region (4000–400 cm^{-1}) by using 16 scans and 4 cm^{-1} resolution. Three spectra were obtained for each sample.

Statistical analysis

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

Data pre-processing and multivariate analysis

Each FTIR spectrum was baseline corrected and smoothed to seven data points,[13] by using a software to minimize the differences between spectra due to the baseline shifts. The spectra were then exported to ASCII file and excel files were prepared for chemometric analysis. The multivariate analyses ((PCA, OPLS-DA, OPLS-DA-HCA and PLS models) of FTIR data were produced using SIMCA-P software version 13 (Umetrics, Umea Sweden).

RESULTS AND DISCUSSION

Most of the accessions showed DPPH free radical scavenging activity comparable to quercetin (standard). The IC_{50} values of extracts (100 µg/mL) ranged from 13.58 ± 3.56 µg/mL to 79.3 ± 3.2 µg/mL, though they were less than that of quercetin (Table 1). In regards to the syconia, only few studies on some varieties of *F. deltoidea* have been found compared to the leaves part such as DPPH radical scavenging activity of syconia of var. deltoidea and var. intermedia (both with IC_{50}; 7.8 µg/mL).[14]

In FRAP assay, the ability of the plant/food extracts to reduce Fe^{3+} to Fe^{2+} were evaluated. The results showed that almost all extracts have strong reducing power. FRAP values of the extracts ranged from 1.1 (M234B) to 9.72 (K003) mmol/g which in comparison indicated significant differences (p<0.05) between all varieties (Table 1). FRAP of var. *angustifolia* and var. *kunstleri* were found to be significantly lower (1.8 and 1.3 mmol/g)[15] than the similar varieties used in our study.

The current study indicated strong α-glucosidase inhibition activity in most of the syconia extracts of the seven *F. deltoidea* varieties. Potential activity of all accessions was detected (IC_{50}) except from var. *motleyana*. The representatives from the seven varieties with highest inhibition are shown in Figure 1. Among all accessions tested, the extracts of var. *trengganuensis* (T002) and var. *kunstleri* (K003) at different concentrations (1.56 to 100 µg/mL) exhibited the strongest activity with IC_{50} of 36.7 and 37.8 µg/mL, respectively (Table 2). Our findings are consistently agreed with[16] who reported ethanol extract of *Ipomoea aquatic* had stronger α-glucosidase inhibition, in concentration dependent manner.

The mean spectra of three different measurements were given in Figure 2. The spectra showed broad peaks at wavenumbers in range 3600-3000 cm^{-1} and sharp peaks at 1604.77 and 1038 cm^{-1}. They suggested the presence of O-H bonds of either alcohols, phenols or water fraction at 3292.2 cm^{-1}, the sp^3 and sp^2 stretching of C-H bonds at bands ranging from 3000 cm^{-1} to 2850 cm^{-1} and the saturated fatty acids fraction at 2923 cm^{-1}.[17] The C=O stretching of aldehyde group presence at 1734.3 cm^{-1},[1] C=C stretching of alkenes and N-H bending of amines and amides at 1612.4 cm^{-1},[1] and the vibration of N=O and stretching of C=C in aromatics at 1514.2 cm^{-1},[1] were also assigned. Besides, other peaks at 1416 cm^{-1} were assigned as the alkane C-H bending, at 1368.6 cm^{-1} represent S=O, at 1162 cm^{-1} for carboxylic acids and 1043.5 cm^{-1} C-N for vibration of amines. In addition, the fingerprints of 1400 to 900 cm^{-1} may also be the carbohydrates signals.[17]

Table 1: DPPH free radical scavenging activity, ferric reducing power (FRAP) of ethanolic extracts of syconia of seven varieties of *Ficus deltoidea*.

Accession Code	DPPH scavenging activity (IC_{50}; µg/mL)	FRAP (mmol Fe^{2+}/g)
T002	31.83 ± 5.48^{cde}	6.13 ± 0.33^{ef}
T019	60.42 ± 2.67^{b}	2.82 ± 0.36^{ij}
T020	ND	2.27 ± 0.21^{jkl}
K003	13.58 ± 3.57^{fg}	9.72 ± 0.70^{a}
K217	18.17 ± 3.21^{efg}	7.91 ± 0.37^{cd}
K313	17.83 ± 3.62^{efg}	7.64 ± 0.43^{cd}
A171	23.83 ± 5.58^{def}	6.47 ± 0.29^{e}
A321	36.50 ± 5.41^{cd}	5.02 ± 0.01^{g}
A295	65.33 ± 13.01^{ab}	2.67 ± 0.23^{ijk}
D006	15.83 ± 3.82^{efg}	8.64 ± 0.64^{b}
D172	14.50 ± 2.18^{fg}	7.39 ± 0.54^{d}
D156	25.17 ± 6.66^{cdef}	8.14 ± 1.05^{bc}
B013	38.42 ± 9.74^{cd}	3.27 ± 0.30^{i}
B014	16.75 ± 2.46^{efg}	7.35 ± 0.35^{d}
B378	22.42 ± 3.02^{defg}	5.50 ± 0.25^{fg}
I323	40.75 ± 1.64^{c}	4.19 ± 0.16^{h}
I386	68.83 ± 6.17^{ab}	2.82 ± 0.24^{ij}
I387	79.33 ± 3.21^{a}	2.73 ± 0.18^{ij}
M234A	77.20 ± 21.77^{a}	1.84 ± 0.28^{l}
M234B	ND	1.11 ± 0.23^{m}
M234C	63.33 ± 24.77^{ab}	1.96 ± 0.09^{kl}
Quercetin	5.50 ± 0.87^{g}	NT

Values are the means ± standard deviation based on three different experiments. Superscript letters refer to significant different (p<0.05) by comparing among syconia accessions. Means with different superscript letters were significantly different (p<0.05). IC_{50}: inhibition concentration. ND: not detected, NT: not tested, T: var. *trengganuensis*, K: var. *kunstleri*, A: var. *angustifolia*, D: var. *deltoidea*, B: var. *bilobata*, I: var. *intermedia*, M: var. *motleyana*.

Table 2: α-glucosidase inhibition of ethanolic extracts of syconia of seven varieties of *Ficus deltoidea*.

Accession Code	α-Glucosidase Inhibition (IC_{50}; µg/mL)
T002	36.75 ± 2.75^{f}
T019	ND
T020	ND
K003	37.67 ± 4.22^{f}
K217	44.33 ± 12.34^{ef}
K313	58.00 ± 17.84^{cdef}
A171	56.17 ± 10.68^{def}
A321	63.75 ± 18.10^{bcde}
A295	ND
D006	81.50 ± 12.26^{abc}
D172	77.83 ± 5.01^{abcd}
D156	71.83 ± 12.92^{abcd}
B013	ND
B014	70.67 ± 12.50^{abcd}
B378	83.83 ± 19.66^{ab}
I323	76.67 ± 1.53^{abcd}
I386	ND
I387	89.50 ± 4.27^{a}
M234A	ND
M234B	ND
M234C	ND
Quercetin	65.17 ± 18.87^{bcde}

Values are the means ± standard deviation based on three different experiments. Superscript letters refer to significant different ($p<0.05$) by comparing among syconia accessions. Means with different superscript letters were significantly different ($p<0.05$). IC_{50}: inhibition 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*.

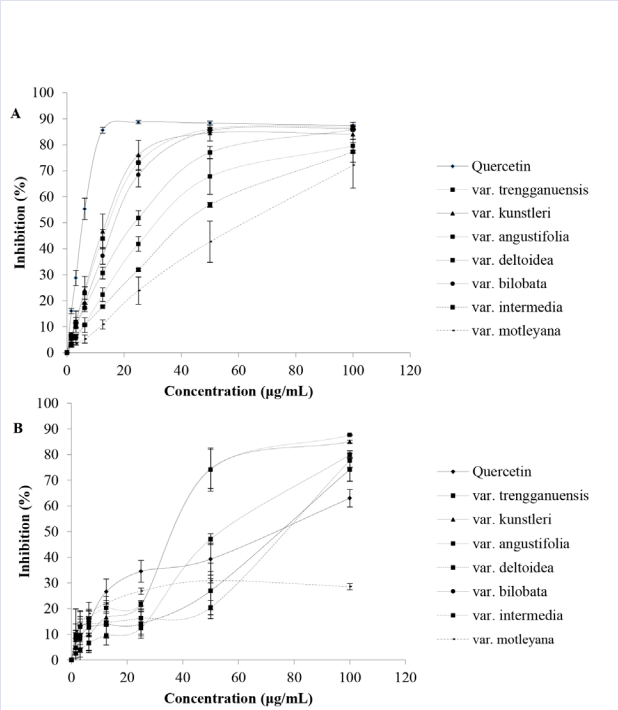

Figure 1: Bioactivities of ethanolic extracts of the seven varieties of *Ficus deltoidea* syconia. A) DPPH free radical scavenging activity B) α-glucosidase inhibition. Data represented by the most active accession of each variety.Values are the means ± standard error based on three different experiments.

FTIR data sets were subjected to principal component analysis (PCA) for unsupervised classification of syconia of the seven varieties of *F. deltoidea*. The first two principal components explained 80.2% of the total variance. The model revealed goodness of fit R^2X (cum) and prediction of the model Q^2 (cum) with values of 0.986 and 0.973, respectively. The loading line plots showed that 2918, 2849, 1730 and 1708 cm^{-1} were the fingerprints contributed to the variation along PC1 Meanwhile, the loading plot along PC2 revealed that 1602 and 1440 cm-1 contributed the most to the variation (data not shown). In the present study, clustering of groups of varieties in PCA model of FTIR spectra were observed, though some of the varieties accessions were overlapped (Figure 3A). The overlapping of spectra was suggested to be due to similarity in their phytochemicals content. Meanwhile, the clusters differences were possibly due to other distinctive metabolites variation such exhibited by Sri Lanka green tea that clustered away from teas of other origins.[18]

Orthogonal partial least square–discriminant analysis (OPLS-DA) model was established as the supervised discrimination of syconia of seven varieties of *F. deltoidea*. The model was confirmed by the Q^2Y and R^2Y cumulatives, in which R^2Y (cum) was 0.964 and Q^2 (cum) was 0.914. The first two PCs explained 39.2% of the variance. Six clusters were identified on PC1. The score plot showed that var. *intermedia* and var. *bilobata* were clustered together, revealing that the two varieties have phytochemicals similarity (Figure 3B). Var. *trengganuensis*, var. *angustifolia* and var. *motleyana,* discriminated in the positive quadrant of PC1, while, var. *intermedia,* var. *bilobata*, var. *kunstleri* and var. *deltoidea* were located into the negative quadrant of PC1, due to fingerprints at 1600, 1515, 1448, 1095 and 762 cm^{-1}. In addition, 1705, 443, 453, 484 cm^{-1} were the fingerprints that contributed to the discrimination of var. *intermedia* and var. *angustifolia* into the positive quadrant of PC2 (data not shown). Interestingly, var. *bilobata* and var. *intermedia* formed one cluster and discriminated themselves from var. *kunstleri* and var. *deltoidea* along PC2. The loading plot reveals that 1729, 1448, 1095, 1705, 443 and 453 cm^{-1} were the fingerprints with the highest contribution to the discrimination ((loading plot data not shown)). OPLS-DA-HCA was performed and similarities among the varieties were calculated using Euclidean distance and the clusters were established using ward hierarchical agglomerative method. The resulting dendrogram of the OPLS-DA shows the clustering of the seven varieties of *F. deltoidea* extracts. Three clusters (C1-C3) were observed. Accessions of var. *trengganuensis* and var. *motleyana* clustered in C1; var. *kunstleri* and var. *deltoidea in* C2; and var. *angustifolia*, var. *intermedia* and var. *var. bilobata* in C3 (Figure 3C). *HCA model* of OPLS-DA gave distinctive clusters involving all accessions of each variety. This indicated that supervised HCA of OPLS-DA model was a better representation than HCA of PCA model in discussing hierarchy of chemicals clusters of the syconia of seven varieties of *F. deltoidea*.

Partial least square (PLS) model was established to investigate the relationship between the bioactivity and FTIR fingerprints of different

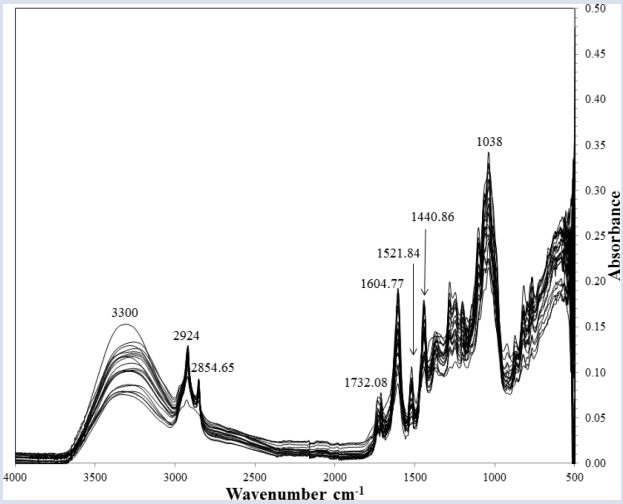

Figure 2: ATR-FTIR spectra of ethanolic extracts of syconia of the seven varieties of *Ficus deltoidea*.

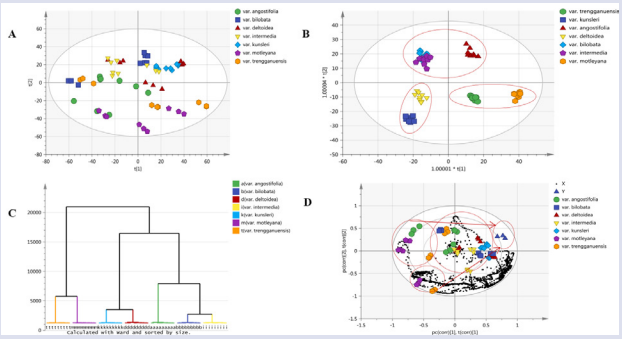

Figure 3: Unsupervised and supervised multivariate data analysis (MVDA) of ATR-IR spectra to discrimination of chemical variability and the relationship between the alpha glucosidse inhibition, antioxidant activity and *Ficus deltoidea* varieties. A) Principal component analysis (PCA), B) Orthogonal partial least square discriminant analysis (OPLS-DA), C) Orthogonal partial least square discriminant analysis hierarchical cluster analysis (OPLS-DA-HCA) and D) Partial least square (PLS) model indicating the relationship between chemicals and extracts bioactivities.

varieties of *F. deltoidea*. PLS model consisted of two variables; the biological activities (DPPH, FRAP and α-glucosidase inhibitory) are represented by the y-axis while the set of wavenumbers are the x-axis (4000-400 cm^{-1}). The PLS model was cross-validated using 100 permutation tests with all Q^2 values lower than those of original ones. The cumulative R^2X, R^2Y and Q^2 values indicated good predictive abilities ($R^2X = 0.904$; $R^2Y = 0.863$; $Q^2 = 0.806$). In the bi-plot, accessions exhibited the most potent biological activities (DPPH, FRAP and α-glucosidase) include var. *kunstleri* (K003, K217, K313), var. *deltoidea* (D006, D172, D156), var. *bilobata* (B014, B378), var. *angustifolia* (A171, A321) and var. *intermedia* (I323, I386, I387); were located on the right side of PC1 which separated from other accessions having weaker biological activities on PC1 left side (Figure 3D). Interestingly, in this study, PLS model of FTIR spectra of syconia of the seven varieties of *F. deltoidea* showed discrimination which corresponded to bioactivity results (Figure 3D).

CONCLUSION

In general, the results show potential α-glucosidase inhibitory and antioxidant activities of syconia extracts of almost all varieties. Among all *Ficus deltoidea* varieties, most promising antioxidant and anti hyperglycemic activities were found in var. *kunstleri* (K003, K217, K313), var. *trengganuensis* (T002) and var. *angustifolia* (A171, A321). OPLS-DA model of FTIR data successfully discriminated the varieties, while PLS model had identified the correlation between var. *kunstleri*, var. *deltoidea* and var. *intermedia* bioactivity and their respective chemicals fingerprints, those were also identified. Thus, their syconia part were determined to be a good source of natural antioxidants as well as α-glucosidase inhibitors. Instead of being underutilized, the syconia of these targeted varieties (elite accessions) could be developed as ingredient in food products and health supplement in local agro-industry besides the widely studied *Ficus deltoidea* leaves.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

ABBREVIATIONS

ATR-IR: Attenuated Total Reflectance-Infrared; **FTIR:** Fourier Transform Infra-Red; **MVDA:** Multi Variate Data Analysis; **DPPH:** 2,2′-diphenylpicryl hydrazyl**; FRAP:** Ferric Reducing Antioxidant power; **PCA:** Principal Component Analysis; **HCA:** Hierarchical Cluster Analysis; **OPLS-DA:** Orthogonal Partial Least Square–Discriminant Analysis; **PLS:** Partial Least Square; **OPLS-DA-HCA:** Orthogonal Partial Least Square–Discriminant Analysis-Hierarchical Cluster Analysis

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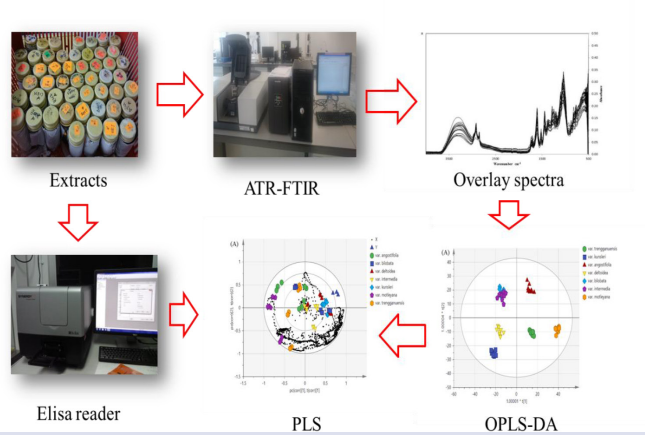
GRAPHICAL ABSTRACT



SUMMARY

- FTIR Metabolomics approach was used to understand the variability of syconia of seven varieties of *Ficus deltoidea*.
- FTIR combine with chemometric successfully discriminated the seven varieties of *Ficus deltoidea*.
- PLS models were developed and identified the correlation between samples and bioactivity and the fingerprints contributed.

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