ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Issue 5, 2014

ISSN - 0974-2441

Research Article

APPLICATION OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY AND FOURIER TRANSFORM INFRARED PROFILING COUPLED WITH CHEMOMETRICS FOR THE DIFFERENTIATION OF THE VARIETIES OF *FICUS DELTOIDEA* JACK

KHAMSAH S MOHD[1]*, AZIERAH AZEMIN[1], MOHD SR HAMIL[2], AMIRUL RA BAKAR[2], SARAVANAN DHARMARAJ[3], MOHAMMAD R HAMDAN[2], HABSAH MOHAMAD[4], NASHRIYAH MAT[1], ZHARI ISMAIL[2]

[1]School of Biotechnology, Faculty of Agriculture, Biotechnology and Food Sciences, University Sultan Zainal Abidin, Tembila Campus, 22200 Besut, Terengganu, Malaysia. [2]Department of Pharmacology, Faculty of Medical and Health Sciences, University Sultan Zainal Abidin, Kota Campus, 20400 Kuala Terengganu, Terengganu, Malaysia. [3]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, University Science Malaysia, 11800 Penang, Malaysia. [4]Section of Natural and Synthetic Product, Institute of Marine Biotechnology, University Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia. Email: khamsahsuryati@unisza.edu.my

Received: 14 July 2014, Received and Accepted: 7 August 2014

ABSTRACT

Objective: The aims of this study were to identify and evaluate the chemical markers of Ficus deltoidea Jack (FD) varieties namely var. deltoidea, var. trengganuensis, var. kunstleri, var. motleyana, var. intermedia, var. borneensis, var. bilobata and var. angustifolia by high performance thin layer chromatography (HPTLC) and to discriminate between the varieties based on Fourier transform infra-red (FTIR) data.

Methods: Methanol extracts of eight varieties and water extracts of five varieties were subjected to HPTLC and FTIR fingerprinting analysis. Quantification of markers (vitexin and isovitexin) was performed by HPTLC. Cluster analysis of principal component analysis (PCA) and hierarchical cluster analysis (HCA) was employed to discriminate between the varieties based on their FTIR data.

Results: Two markers, vitexin and isovitexin, were found in all of the varieties, but their contents were varied in the different samples. Cluster analysis (PCA and HCA) was employed to discriminate between the varieties based on their FTIR data. Clear separation between the methanol extracts of the varieties was observed, whereas the discrimination of the water extracts of the varieties showed some disparities.

Conclusion: The findings indicate that HPTLC combined with FTIR coupled with chemometric analysis is a reliable analytical tool for the identification and discrimination of FD varieties.

Keywords: Ficus deltoidea Jack, High performance thin layer chromatography, Fourier transform infra-red profiling, Vitexin, Isovitexin.

INTRODUCTION

Ficus deltoidea (FD), or mistletoe fig, which is commonly consumed as a tea, is a herb with a variety of therapeutic potentials. Traditional practitioners use this plant in herbal remedies to treat several illnesses, such as headaches, hypertension and hyperglycemia [1,2]. Studies have shown that FD leaves have several biological activities, such as antioxidant and neuroprotection activities [3,4]. This plant has received great attention in recent years due to pharmacological evidence of its anti-diabetic properties [5-7]. In terms of chemical components, recent reports have characterized at least 25 flavonoids, and the main constituents have been identified to be flavan-3-ol monomer, proanthocyanidins, and C-linked flavone glycosides [8,9], which have been found to be responsible for 85% of the total antioxidant activity of the leaves. FD leaves have also been reported to possess phenolic and flavonoid compounds, which are also found in black and green teas [8,10]. The C-linked glycoside flavones vitexin and isovitexin have been shown to inhibit alpha-glucosidase [11], an enzyme that is responsible for the breakdown of carbohydrate to sugar. Taking its therapeutic potential into account, it is worth developing this plant as an herbal medicine. However, the variability of the plant in terms of leaf morphology is tricky, especially for inexperienced collectors. Traditional practitioners classify FD into more than 20 varieties based on the morphology of the leaf and stem and the growing habitats. Misidentification is highly possible due to its extreme morphological variations and the unclear boundaries between varieties. The leaf characteristics are likely the most variable and show heterophylly [12,13]. The taxonomic classification performed by Musa [14] grouped FD into only six varieties, namely var. bilobata, angustifolia, intermedia, kunstleri, motleyana and trengganuensis. Fatihah et al. [15] made the first attempt to dissect a systematic relationship between seven varieties of FD from peninsular Malaysia using a morphological phylogenetic framework. These researchers found that the FD specimens found in the Malaysian region could be classified into two subspecies: Subspecies deltoidea consisted of var. deltoidea, var. bilobata, var. angustifolia, var. kunstleri and var. trengganuensis, whereas subspecies motleyana comprised var. intermedia and var. motleyana.

With the confusion arises from the varietal status and the complexities of the chemical compounds, it is justified to identify the uncomplicated way to assess the identity and therapeutic quality of this plant in order to be used as herbal medicine. The uses of chromatographic techniques such as high performance thin layer chromatography (HPTLC) and HPLC and spectroscopic fingerprints such as Fourier transform infra-red (FTIR) are commonly applied in the analysis of plant authentication and quality control [16,17]. To provide a fingerprint, the entire chromatogram may be used as a chemical profile, or a chromatographic discriminating region or a selection for significant chemical markers may be used [18].

The aim of this study was to identify and evaluate the chemical markers and differentiates the varieties of FD through a discrimination of their metabolites via principal component analysis (PCA) and hierarchical cluster analysis (HCA). The evaluation of identification was carried out by HPTLC using the C-flavonoid glycosides vitexin and isovitexin as the standard markers. The metabolomics data for discriminant analysis was acquired using FTIR. The results from the fingerprinting studies will be useful for differentiating between varieties.

METHODS

Chemicals and reagents

The pure compounds vitexin and isovitexin were purchased from Fluka-Sigma. The solvents methanol, ethyl acetate, and formic acid are products of Merck (Damstadt, Germany). HPTLC plates precoated with silica gel F_{254} (layer thickness of 0.2 mm) were purchased from Merck.

Instrumentations

HPTLC

HPTLC protocol was adapted from [19] with some modification. The extracts were spotted on HPTLC plates precoated with silica gel F_{254} (layer thickness of 0.2 mm) (Merck) using Camag Linomat 5 model. The samples were streaked in the form of narrow bands with a length of 8.0 mm 8 mm from the bottom edge, 40 mm from the margin, and 13.3 mm apart at a constant rate of 100 nL/seconds using a nitrogen aspirator. The migration distance was 8 cm with a migration time of 25 minutes. The densitometric analysis of the separated components was carried out using a Camag thin layer chromatography (TLC) scanner 3 (Camag, Switzerland) in the absorbance mode at 340 nm. The bands were scanned using deuterium and tungsten lamps, and the scanning speed was maintained at 20 mm/seconds with a macro slit dimension of 8.00 mm × 0.2 mm. The integration of chromatograms was performed using the Camag TLC scanner system and win CATS software (Camag, Switzerland).

Selection of mobile phase for HPTLC

Several solvent systems for vitexin and isovitexin were screened. The selection of the solvent system was based on the separation of marker compounds. The mobile phase was ethyl acetate:formic acid (0.1%):methanol (5:5:2 v/v/v). The developed plate for each FD extract was visualized under a wavelength of 254 nm and was further scanned for 3D densitogram and ultraviolet spectrum analysis at 340 nm.

Standard curve of markers

Appropriate volumes of the standard solutions (100 µg/ml) for both vitexin and isovitexin were prepared in methanol and were spotted on HPTLC plates. A different volume of the stock solution of each standard marker (1, 1.5, 2, 2.5 and 3 µl) was spotted on HPTLC plates to obtain 100, 150, 200, 250 and 300 ng of the standard marker per spot, respectively, for vitexin and isovitexin. The calibration curve was plotted using the peak areas against the corresponding concentrations.

Accuracy and precision of the assay

The accuracy of the assay was tested at 150, 200 and 300 ng of vitexin and isovitexin. The concentration of the markers in the extracts (n=5) at each level was compared with the theoretical concentration. The intra-day precision was evaluated by repeatedly analyzing samples with concentrations of 150, 200 and 300 ng of the markers per spot (n=5). The inter-day precision analysis was similarly evaluated by repeated analysis of samples with concentrations of 150, 200, and 300 ng of both markers (per spot) once daily for 5 days.

FTIR

The FTIR spectra were obtained and recorded using a Perkin Elmer spectrum 400 infrared (IR) spectroscopes coupled with air-cooled deuterated triglycine sulfate. The attenuated total reflectance scan technique was used directly for all of the samples. The IR measurements were made at a resolution of 4/cm, and 16 inferograms were co-added before the Fourier transformation. The data were recorded at the mid-IR range of 4000-600/cm.

Data analysis for spectroscopic fingerprinting

To minimize the differences between spectra due to baseline shifts, each FTIR spectrum were baseline corrected using the Spectrum (Perkin Elmer, Inc.) software. The most intense band of peak absorbance was normalized to set normality. The spectra were then exported as a Spectrum. SP file and imported into the multivariate statistical software program. The Unscrambler X (CAMO, Trondheim, Norway). The PCA and HCA classifications were performed using Unscrambler. PCA and HCA were used to discriminate the metabolome of FD varieties.

Sample collection

Plant materials are consisting of leaves of FD var. deltoidea, var. kunstleri, var. angustifolia, var. motleyana, var. bilobata and var. trengganuensis were obtained from University Sultan Zainal Abidin (UniSZA) living collection, Gong Badak Campus, Kuala Terengganu. FD var. intermedia was collected from mount Brinchang, Cameron highland and var. borneensis was collected in Santubong, Sarawak, East Malaysia. Each sample was identified by Prof. Nashriyah Mat, UniSZA, Terengganu, Malaysia and deposited at UniSZA's herbarium.

Preparation of plant extracts

For the methanol extract, 1 g of the powder sample was macerated with 19 ml of methanol for 3 days. For the water extract, 1 g of powder material was extracted at 100°C with distilled water for 1 hr. The filtered extracts were dried using a rotary evaporator and maintained at −20°C prior to analysis.

Preparation of sample extract for quantification

Samples of each variety of FD were prepared at a concentration of 60 mg/ml in methanol. The volume of sample extracts (n=3) applied was 2 µl to obtain 120,000 ng in each spot. The mass of the standard markers in the samples was calculated using calibration curves.

RESULTS AND DISCUSSION

In this work, eight authenticated varieties of FD was analyzed. The varieties, namely var. deltoidea (specimen no: FD 007), var. bilobata (specimen no: FD 013), var. angustifolia (specimen no: FD 171) var. kunstleri (specimen no: FD 009), var. motleyana (specimen no: FD 258) and var. trengganuensis (specimen no: FD 090), were obtained from the UniSZA living collection. The sample of var. intermedia (specimen no: FD-CH-1) was collected from the forest reserve, Mount Brinchang, Cameron Highland. The sample of var. borneensis (specimen no: FD 260) was collected at Santubong, Sarawak. Herbarium specimens for all eight varieties were deposited at the Herbarium Faculty of Agriculture, Biotechnology and Food Sciences, UniSZA. Methanol extracts of all varieties were analyzed by HPTLC and FTIR. Due to the sample availability, only five water extracts were obtained and analyzed by HPTLC and FTIR.

To examine the compositional differences between FD varieties, TLC analysis was performed. The TLC profiles of methanol and water extracts of the FD varieties are shown in Fig. 1a-d, respectively. The results indicate that all of the sample constituents are clearly separated without any tailing and diffuseness using the solvent system of ethyl acetate:Formic acid (0.1%):Methanol (5:5:2 v/v/v). Two compounds, namely vitexin and isovitexin, were used as markers. The typical chromatogram of FD showed two bands of vitexin and isovitexin with relative R_f values of 0.46 and 0.36, respectively. All of the samples showed a dark band when visualized under 254 nm, and this band represents glycoside flavones due to the quenching of double bonds in the compound in the methanol and water extracts. The intensities of vitexin and isovitexin varied between the varieties. The methanol extracts showed a higher intensity of these compounds compared to the water extracts. As shown in the TLC chromatogram presented in Fig. 1, the excitation of isovitexin in var. borneensis at 254 nm was found to be the strongest, and vitexin appears to exhibit its highest intensity in var. deltoidea. The HPTLC fingerprint profiles of the methanol and water extracts of all of the varieties are shown in Fig. 1b and d, and these profiles indicate the occurrence of eight components in the methanol extracts and five components in the water extracts.

The quantitative analysis of vitexin (Fig. 2a) and isovitexin (Fig. 2c) based on the densitometry measurement of the peaks was performed using the HPTLC technique. HPTLC has emerged as an efficient tool for the quantitative evaluation of herbal drugs [20] due to its simplicity and minimum sample clean-up requirement. All standard curves were linear over the range of 100-300 ng. The standard curve is shown

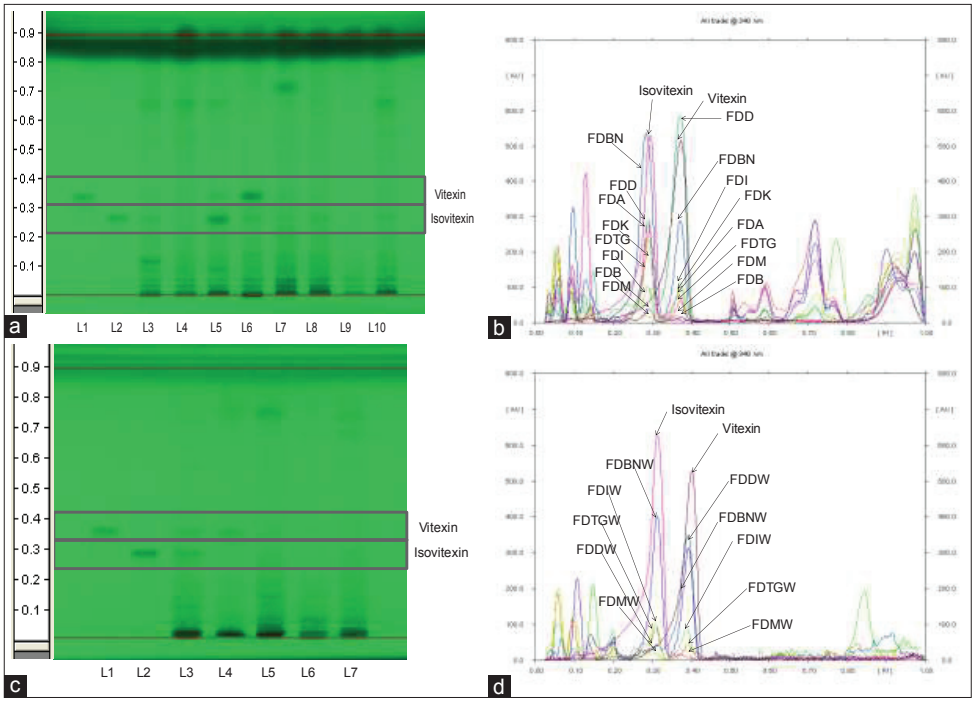

Fig. 1: Methanol extracts of *Ficus deltoidea* (FD) leaves and marker compounds (vitexin and isovitexin) were spotted on pre-coated silica gel 60 F_{254} high performance thin layer chromatography (HPTLC) plates. (a) Thin layer chromatography (TLC) chromatogram of the methanol extracts of all varieties of FD visualized at 254 nm, (b) HPTLC fingerprinting of the methanol extracts of all varieties of FD, (c) TLC chromatogram of the water extracts of all varieties of FD visualized at 254 nm, (d) HPTLC fingerprinting of the water extracts of all varieties of FD. L1: vitexin; L2: isovitexin; methanol extract; L3: FD var. *angustifolia*, L4: FD var. *bilobata*, L5: FD var. *borneensis*, L6: FD var. *deltoidea*, L7: FD var. *intermedia*, L8: FD var. *motleyana*, L9: FD var. *trengganuensis*, L10: FD var. *kunstleri*. Water extract; L3: FD var. *borneensis*, L4: FD var. *deltoidea*, L5: FD var. *intermedia* L6: FD var. *motleyana*; L7: FD var. *trengganuensis*. FDA: FD var. *angustifolia*; FDB: FD var. *bilobata*; FDBN/FDBNW: FD var. *borneensis*; FDD/FDDW: FD var. *deltoidea*; FDI/FDIW: FD var. *intermedia*; FDK: FD var. *kunstleri*; FDM/FDMW: FD var. *motleyana*; FDTG/FDTGW: FD var. *trengganuensis*

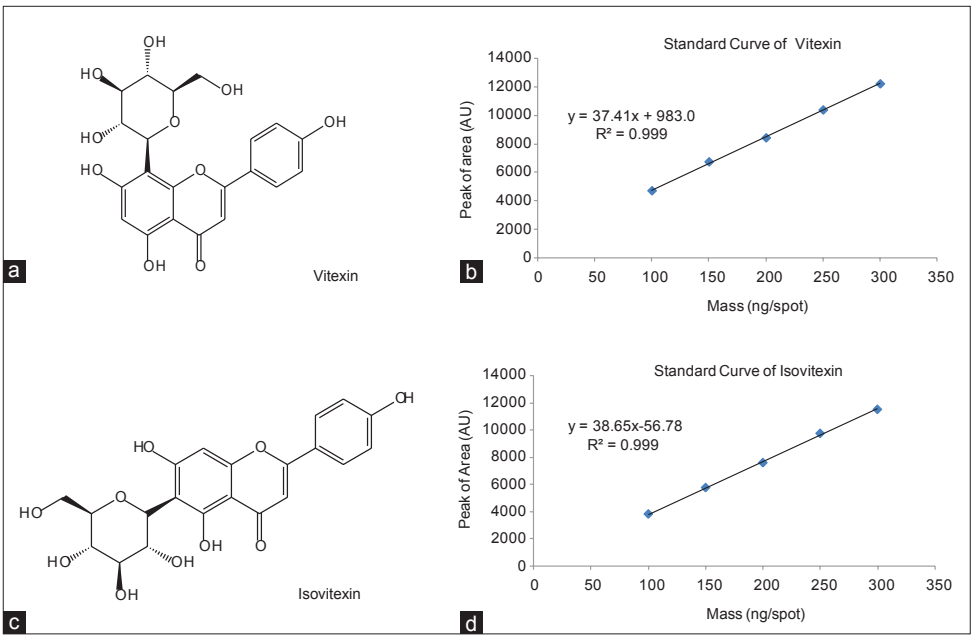

Fig. 2: Structure of (a) vitexin and (c) isovitexin. Standard curve for (b) vitexin and (d) isovitexin by high performance thin layer chromatography

in Fig. 2b and d. The linear regression for the standard markers vitexin and isovitexin were R^2 = 0.999. The limits of detection for vitexin and isovitexin were 22.6 and 44.9 ng, respectively, and the limits of quantification were 68.5 and 136 ng, respectively. The % relative standard deviation <3% for both intra-day and inter-day revealed the excellent accuracy and high precision of the assay method. The low coefficient of variation was indicative of the acceptable inter-day and intra-day precision of the assay. The recoveries of vitexin and isovitexin were 101.71% and 91.63%, respectively. The intensities of vitexin and isovitexin also varied between the varieties as shown in Fig. 3. It was

found that FD var. deltoidea contains the highest amount of vitexin, whereas FD var. borneensis contains the most amount of isovitexin (Table 1).

The HPTLC fingerprinting assay developed in this study is specific to the selected solvent system and can be used to differentiate between varieties of FD. The R_f value of the markers can be a useful tool to provide sufficient information for the identification, authentication and standardization of FD.

In the present study, FTIR was used to discriminate FD varieties. The typical FTIR spectra for the methanol and water extracts of different varieties of FD are shown in Fig. 4. The spectra of all samples looked identical. The number of peaks was generally more or less the same. A

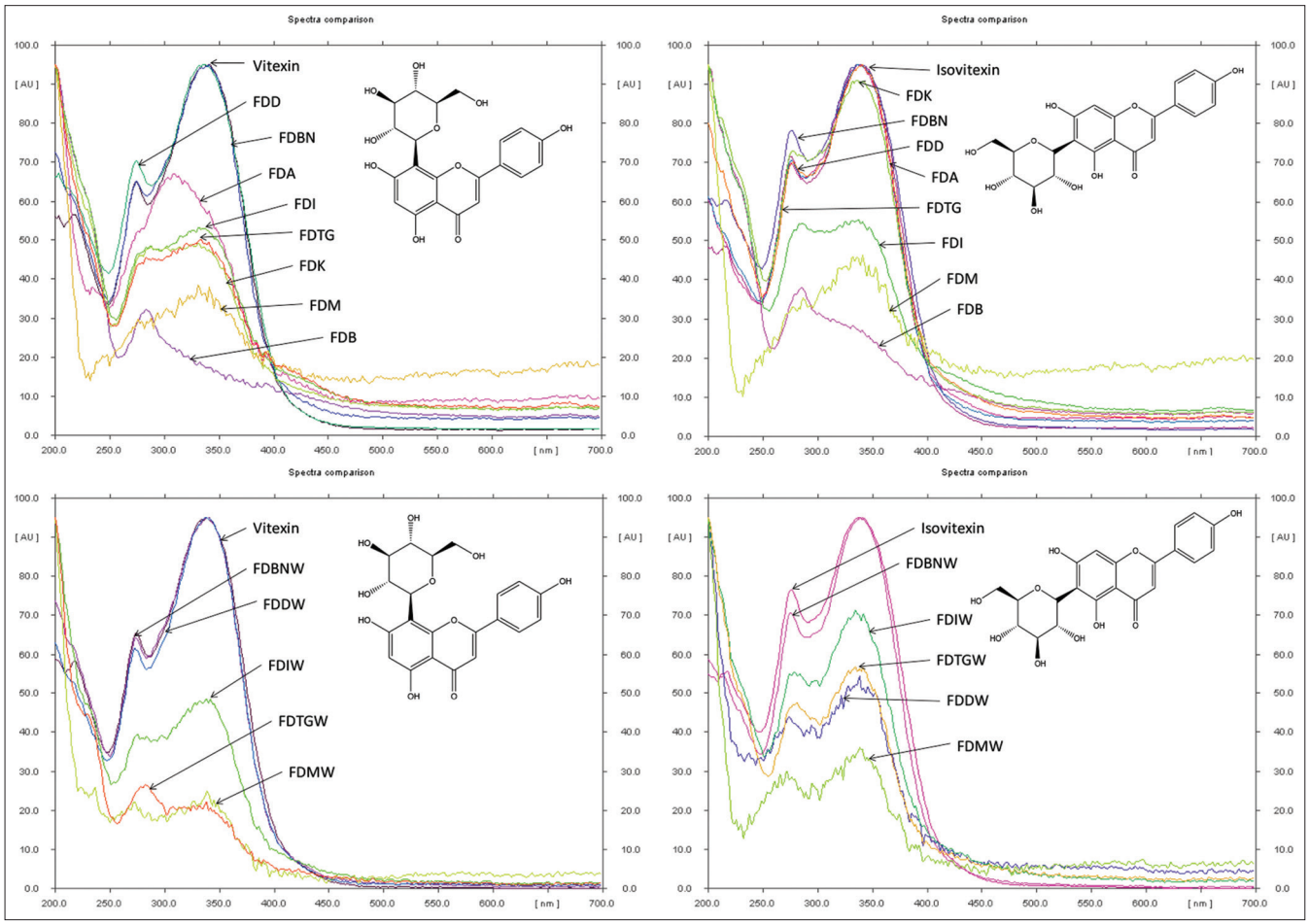

Fig. 3: UV-VIS spectra of vitexin and isovitexin in the methanol and water extracts of all FD varieties. The intensities of vitexin and isovitexin were varied in the methanol (a and b) and water (c and d) extracts of the different varieties. FDA: FD var. angustifolia; FDB: FD var. bilobata; FDBN/FDBNW: FD var. borneensis; FDD/FDDW: FD var. deltoidea; FDI/FDIW: FD var. intermedia; FDK: FD var. kunstleri; FDM/FDMW: FD var. motleyana; FDTG/FDTGW: FD var. trengganuensis

Table 1: Quantification data of vitexin and isovitexin in methanol and water extracts of *Ficus deltoidea*

Quantification of vitexin and isovitexin in FD (ng/µg)				
FD varieties	**Vitexin**	**Percentage in extract**	**Isovitexin**	**Percentage in extract**
Methanol extracts				
var. angustifolia	0.0008±2.51	0.08	0.0008±2.51	0.22
var. bilobata	0.0003±3.10	0.03	0.0008±2.51	0.06
var. bornensis	0.0008±2.16	0.25	0.0008±2.51	0.50
var. deltoidea	0.0008±0.66	0.60	0.0008±2.51	0.32
var. kunstleri	0.0008±3.91	0.12	0.0008±2.51	0.08
var. intermedia	0.0008±1.31	0.13	0.0008±2.51	0.23
var. motleyana	0.0008±2.00	0.03	0.0008±2.51	0.07
var. trengganuensis	0.0008±4.03	0.03	0.0008±2.51	0.15
Water extract				
var. bornensis	0.0008±2.51	0.30	0.0008±2.51	0.47
var. deltoidea	0.0008±2.51	0.59	0.0008±2.51	0.21
var. intermedia	0.0008±2.51	0.08	0.0008±2.51	0.19
var. motleyana	0.0008±2.51	0.003	0.0008±2.51	0.11
var. trengganuensis	0.0008±2.51	0.02	0.0008±2.51	0.15

FD: Ficus deltoidea

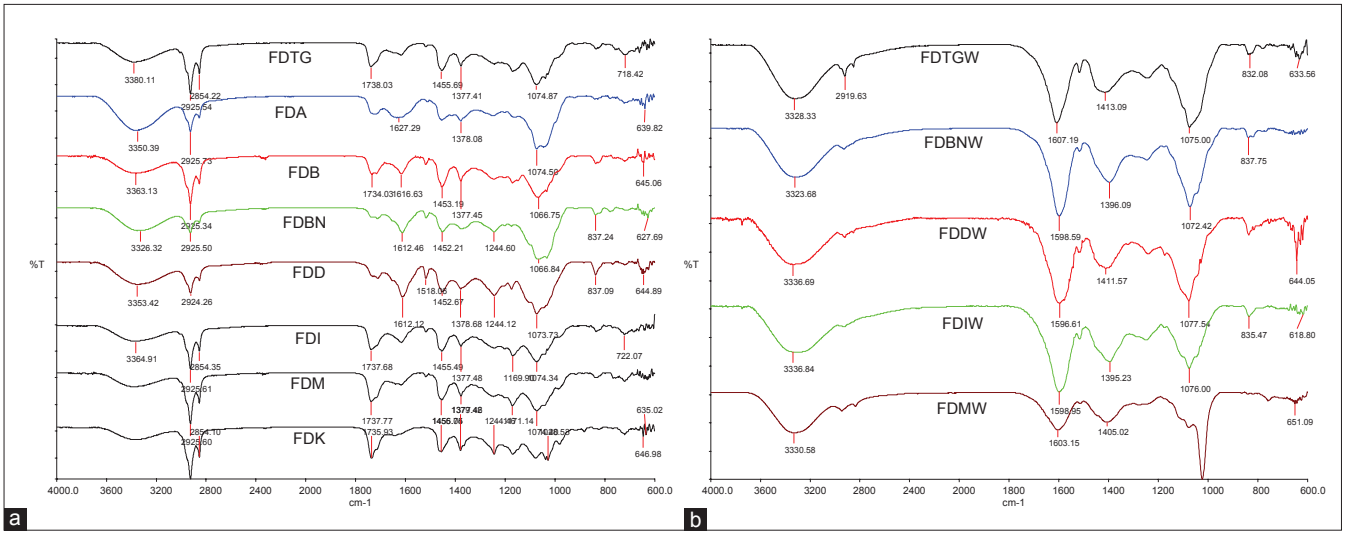

Fig. 4: Fourier transforms infra-red spectra of the methanol and water extracts of the different varieties of *Ficus deltoidea* (FD). FDA: FD var. *angustifolia*; FDB: FD var. *bilobata*; FDBN/FDBNW: FD var. *borneensis*; FDD/FDDW: FD var. *deltoidea*; FDI/FDIW: FD var. *intermedia*; FDK: FD var. *kunstleri*; FDM/FDMW: FD var. *motleyana*; FDTG/FDTGW: FD var. *trengganuensis*

similar result was found for the intensity of the peaks, although there were some peaks whose presence and intensity varied greatly even within the same variety. It was found that the methanol and water extracts exhibited different fingerprints. These data are in agreement with the HPTLC profiles, where more bands were observed in the methanol extracts compared with the water extracts. The FTIR peaks are attributed to stretching and bending vibrations that characterize the functional groups. Because the FTIR literature data on FD is very limited, a direct comparison cannot be performed. As shown in the overall FTIR spectra of the methanol extracts, thirteen major peaks exist, and a similar finding was obtained for all of the varieties. The following peaks were found to be the most stable in the spectrum: The peaks at 3550-3200 cm^{-1} are assigned to intermolecular hydrogen bonds, the peaks at 2930-2850/cm are assigned to the asymmetric stretching of methylene (v_{as} CH_2), the peak at approximately 2925/cm is assigned to the symmetric stretching of methylene (v_{as} CH_2), and the peak at approximately 2854/cm [21]. The peaks at 1750-1735/cm are assigned to the C=O stretching vibration of saturated aliphatic ester, the peaks at 1660-1610/cm are assigned to the C=C stretching vibration of vinyl ether, and the peaks at 1570-1515/cm are assigned to secondary acyclic amides resulting from the interaction between the N-H bending and C-N stretching of the C-N-H group. In addition, the asymmetrical bending vibration of C-H bonds (δ_{as} CH_3) occurs near 1450/cm, whereas the symmetrical bending vibration of C-H bonds (δ_s CH_3) appears near 1375/cm. The peaks at 1275-1200/cm are attributed to asymmetrical C-O-C stretching vibration, 1210-1163/cm assigned as C-O stretching vibration for C-C(=O)-O of saturated ester, the peaks at 1075-1020/cm are attributed to symmetrical C-O-C stretching vibration, and the peaks at 900-675/cm are assigned to out-of-plane C-H bending vibration of a ring hydrogen atom strongly coupled to adjacent hydrogen atoms. The methylene rocking vibration (ρ CH_2) of straight-chain alkanes of seven or more carbon atoms appears at approximately 720/cm, and the peaks at 700-610/cm are assigned to the C-H bending vibration of alkynes. In contrast, for the water extracts, eight similar peaks were observed in the spectra of the different varieties. The peaks at 3550-3200/cm are assigned to intermolecular hydrogen bonds, the peaks at 1600-1585/cm and 1500-1400/cm are assigned to the skeletal vibration of mononuclear aromatic hydrocarbons involving C-C stretching within the ring, the peaks at 1570-1515/cm are assigned to the interaction between the N-H bending and C-N stretching of the C-N-H group of secondary acyclic amides, the peaks at 1275-1200/cm are attributed to asymmetrical C-O-C stretching vibration, the peaks at 1075-1020/cm are attributed to symmetrical C-O-C stretching vibration, the peaks at 900-675/cm are assigned to the out-of-plane C-H bending vibration of a ring hydrogen atom, the peaks near 720/cm are assigned to the methylene rocking vibration (ρ CH_2) of straight-chain alkanes, and the peaks at 700-610/cm are assigned to the C-H bending vibration of alkynes. Thus, the significant difference between the methanol and water extracts is the absence of peaks at 2930-2850/cm, 1750-1735/cm, 1660-1610/cm, 1450/cm, 1375/cm, 1210-1163/cm and 720/cm in the water extracts and the occurrence of peaks at 1600-1585 and 1500-1400/cm in the water extracts that are absent in the methanol extracts.

The analysis of the spectra of eight varieties of FD leaves revealed that all 13 major peaks are present, with the exception of the peaks at 1570-1515/cm, which are absent in the spectra of var. FD kunstleri (FDK) and var. FD trengganuensis (FDTG). The peaks at 1660-1610/cm were also absent in FDK. In addition, the water extracts of five varieties of FD leaves show the occurrence of all eight major peaks mentioned earlier. From the qualitative point of view, the main differences between the samples were observed in the region between 1800 and 1300/cm, which is known as the "fingerprint" region.

PCA was performed to establish the similarities or the differences between the methanol extracts of eight varieties of FD and the water extracts of five varieties of FD. The raw datasets were obtained from the FTIR spectra. The data were pre-processed using the maximum normalization and the spectral rotation by transforming the transmittance reading to the absorbance using The Unscrambler X software (Camo, Norway). The analysis was based on a 48 × 3311 data matrix assembled such that each row corresponded to the sample and each column represented the spectral data at a given wavelength. From the PCA analysis, the first two components accounted for 97% of the total variance for both extracts. For the methanol extracts, PC1 accounted for 86% of the total variance and provided separation of the evaluated samples into two groups: FDA/FDD/FDBN (positive PC1) and FDTG/FDM/FDB/FDK/FDI (negative PC1) (Fig. 5a). For the water extracts, PC1 accounted for 76% of the total variance and provided separation of the sample into two groups: FDMW/FDTGW/FDBNW/FDIW (positive PC1) and FDDW (negative PC1) (Fig. 5c). Taken as a whole, the peaks that exhibited varied intensity contributed to the clustering observed with PCA. With the use of only two principal components (PC1 and PC2) on the 2D-scatter plot, the eight varieties of FD was significantly clustered together in eight groups. However, the FDI, FDTG and FDB of the methanol extracts were very close and appeared to overlap slightly. This overlap was also observed in the cluster between FDK and FDM. To obtain better discrimination, particularly in these overlapping groups, a 3D-scatter plot was constructed. As shown in Figure 5b, all eight groups are clearly segregated without overlapping.

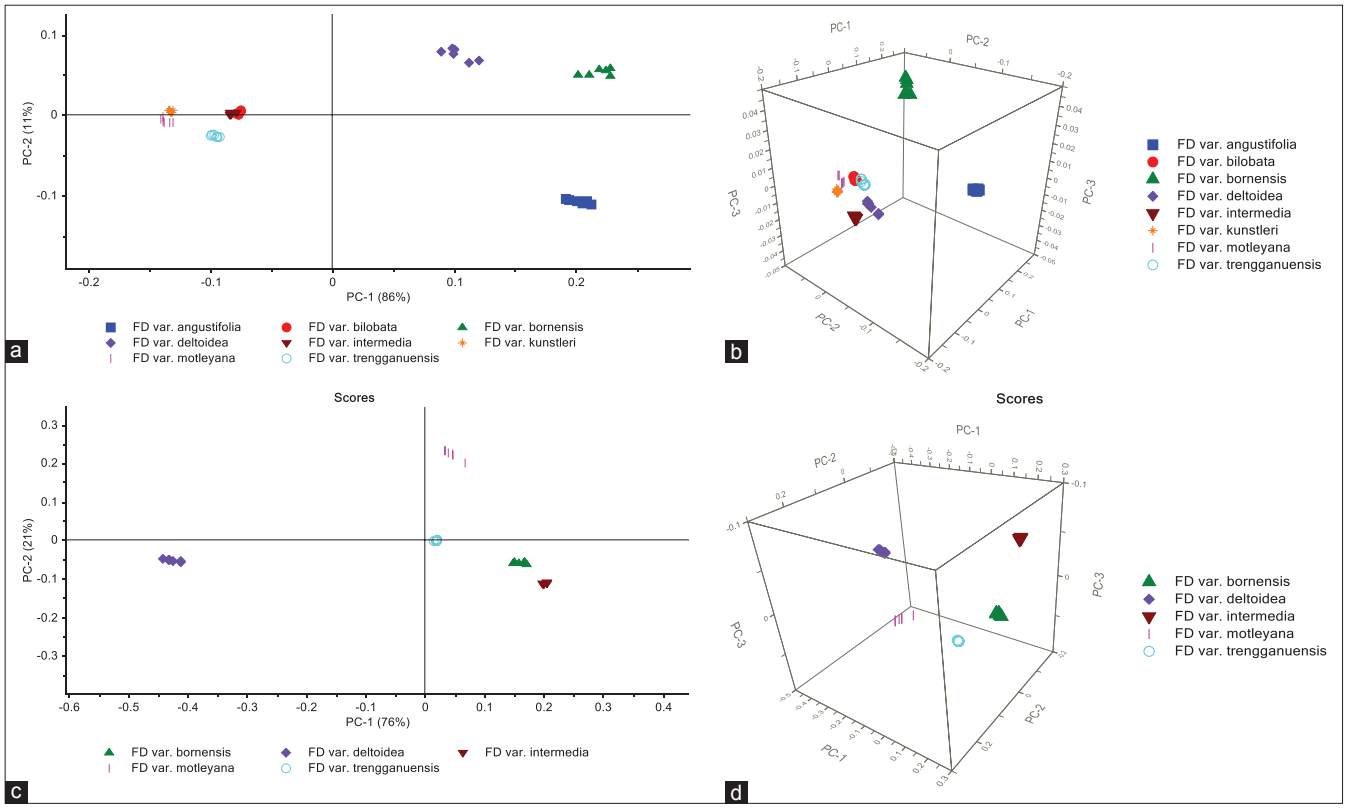

Fig. 5: Principal component analysis principal component analysis scatter plots of the methanol and water extracts of *Ficus deltoidea* (FD). (a) 2D-scatter and (b) 3D-scatter plots of the methanol extracts of FD (c) 2D-scatter and (d) 3D-scatter plots of the water extracts of FD

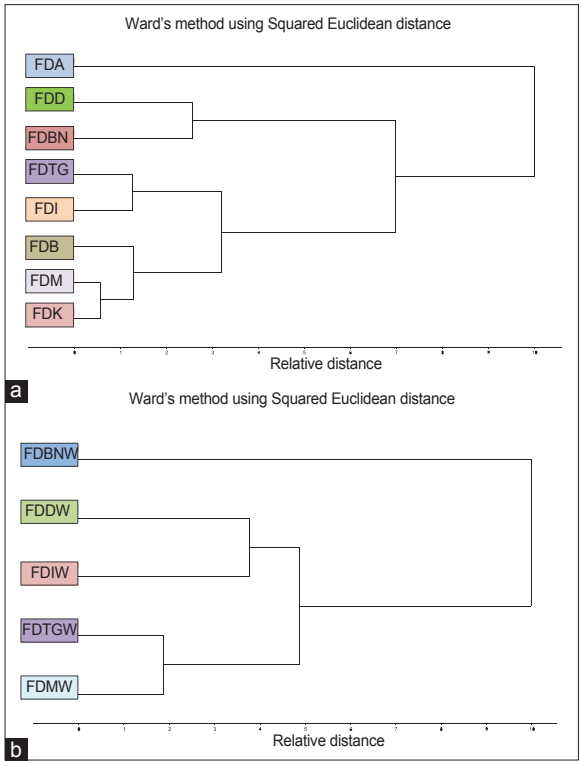

Fig. 6: (a) Hierarchical cluster analysis (HCA) dendrograms of the methanol extracts of *Ficus deltoidea* (FD). (b) HCA dendrograms of the water extracts of FD

To verify the dissimilarity between the varieties, HCA was applied. Ward's hierarchical clustering method and the square of the Euclidean distance were employed to establish the clusters and calculate the dissimilarity coefficients, respectively. The resulting dendrograms are shown in Fig. 6. The group clustering confirmed the PC analysis results. In the case of the methanol extracts, FD can be grouped into two major clusters: The first cluster only consists of FDA, and the second group was divided into two subgroups, namely FDD/FDBN and FDTG/FDB/FDI/FDM/FDK (Fig. 6a). In the case of the water extracts, clear separation between the varieties can be observed into two major clusters: FDDW and FDTGW/FDMW/FDI/FDBNW (Fig. 6b). A classification based on the methanol extracts differs from that obtained with the water extracts. These data corroborate the HPTLC findings, which showed that more chemical constituents are observed in the methanol extracts. However, in any case, FTIR combined with chemometric analysis clearly discriminate the varieties of FD Jack.

CONCLUSION

The use of HPTLC in this work provides a simple, accurate and reproducible quantitative analysis for differentiating the varieties of the studied plant material. This system provides a powerful, flexible and inexpensive separation technique for the analysis of multiple samples at any given time. TLC remains the forerunner for obtaining the first characteristic fingerprint profile of an herbal sample. Thus, to assist the differentiation of the varieties of FD, the HPTLC technique was employed. Two compounds, namely isovitexin and vitexin, were suitable for showing the authenticity, identity and quality of the FD varieties. The employment of chemometric analysis to differentiate plant cultivars, varieties or parts has increased during the past decade. This study showed that it was possible to discriminate between varieties of FD based on a chemometric analysis of spectroscopic data. Despite the minimal pre-processing of the datasets, good clustering was obtained, and the results from PCA and HCA were found to be consistent. The developed chemometric profile and fingerprinting derived in this study can be used as a template to study other plants with similar varieties that exhibit high potential to be developed as new herbal medicines.

ACKNOWLEDGMENTS

The authors are very grateful to the Department of Forestry of Peninsular Malaysia, Sarawak Forestry Corporation and Sarawak Biodiversity Centre for granting the sampling permits. The authors also thank A. Ahmad for the invaluable comments regarding the multivariate analysis. This study was supported by the research acculturation collaborative effort (RACE) Grant Scheme (No. RACE/F1/SG2/UniSZA/1) of the Ministry of Education of Malaysia and the University Grant Scheme of Universiti Sultan Zainal Abidin of Kuala Terengganu, Malaysia (No. UniSZA/12/GU [004]).

REFERENCES

1. Musa Y. Evaluation of growth performance and yield potential of selected emas cotek (Ficus deltoidea) accessions on bris soils. J Trop Agric Food Sci 2006;34(2):229-35.
2. Adam Z, Hamid M, Ismail I, Khamis S, Marsidi N. Antihyperglycemic and glucose tolerance activity of Ficus deltoidea ethanolic extract in diabetic rats. J Sains Kesihatan Malaysia 2010;8(1):25-30.
3. Hakiman M, Maziah M. Non enzymatic and enzymatic antioxidant activities in aqueous extract of different Ficus deltoidea accessions. J Med Plants Res 2009;3(3):120-31.
4. Dzolin S, Aris SS, Ahmad R, Mat ZM. Radical scavenging and neurotoxicity of four variaties of Ficus deltoidea. International Conference on Science and Social Research (CSSR), Kuala Lumpur, Malaysia; 2010. p. 5-7.
5. Farsi E, Shafaei A, Hor YS, Khadeer AB, Yam MF, Attitalla IH, et al. Correlation between enzymes inhibitory effects and antioxidant activities of standardized fractions of methanolic extract obtained from Ficus deltoidea leaves. Afr J Biotechnol 2011;10(67):15184-94.
6. Adam Z, Khamis S, Ismail A, Hamid M. Ficus deltoidea: A potential alternative medicine for diabetes mellitus. Evid Based Complement Alternat Med. 2012;2012:632763.
7. Adam Z, Khamis S, Ismail A, Hamid M. Inhibitory properties of Ficus deltoidea on α-glucosidase activity. Res J Med Plant 2010;4(2):61-75.
8. Omar MH, Mullen W, Crozier A. Identification of proanthocyanidin dimers and trimers, flavone C-Glycosides, and antioxidants in Ficus deltoidea, a Malaysian herbal tea. J Agric Food Chem 2011;59:1363-9.
9. Zunoliza A, Hussain K, Ismail Z, and Mat Ali R. Anti-inflammatory activity of standardized extracts of leaves of three varieties of Ficus deltoidea. Int J Pharm Clin Res 2009;1(3):100-5.
10. Bravo L, Goya L, Lecumberri E. LC/MS characterization of phenolic constituents of mate (Ilex paraguariensis, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. Food Res Int 2007;40:393-405.
11. Choo CY, Sulong NY, Man F, Wong TW. Vitexin and isovitexin from the leaves of Ficus deltoidea with in-vivo a-glucosidase inhibition. J Ethnopharmacol 2012;142(3):776-81.
12. Awang NA, Hasan ZS, Shafie MS. Morphological study of Ficus deltoidea Jack in Malaysia. J Agric Sci Technol 2013;3:144-50.
13. Mat N, Rosni NA, Rashid AN, Haron N, Mohd Nor Z, Hasan NN, et al. Leaf morphological variations and heterophylly in Ficus deltoidea Jack (Moraceae). Sains Malaysiana 2012;41(5):527-38.
14. Musa Y. Variability in morphology and agronomy of emas cotek accesions found in Kelantan and Terengganu. Bul Teknol Tanaman 2005;2:35-48.
15. Fatihah HN, Mat N, Zaimah AR, Zuhailah MN, Norhaslinda H, Khairil M, et al. Morphological phylogenetic analysis of seven varieties of Ficus deltoidea Jack from the Malay Peninsula of Malaysia. PLoS One 2012;7(12):e52441.
16. Janakiraman N, Sahaya SS, Johnson M. UV-VIS and FTIR spectroscopies studies on Peristrophe bicalyculata (Retz.) Nees. Asian J Pharm Clin Res 2011;4(4): 125-9.
17. Yamunadevi M, Mesely EG, Johnson MA. FTIR spectroscopic studies on Aerva lanata (L.) Juss.ex Schult. Asian J Pharm Clin Res 2012;5(2):82-6.
18. Gad HA, El-Ahmady SH, Abou-Shoer MI, Al-Azizi MM. Application of chemometrics in authentication of herbal medicines: A review. Phytochem Anal 2013;24(1):1-24.
19. Shafaei A, Farsi E, Ismail Z, Asmawi MZ. Quantitative high performance thin-layer chromatography method for analysis of vitexin and isovitexin in extracts of leaves of Ficus deltoidea. Asian J Chem 2012;24(5):2283-6.
20. Abdullah Z, Hussain K, Zhari I, Rasadah MA, Mazura P, Jamaludin F, et al. Evaluation of extracts of leaf of three Ficus deltoidea varieties for antioxidant activities and secondary metabolites. Pharmacog Res 2009;1:216-23.
21. Silverstein RM, Webster FX, Kiemle DJ. Spectrometric Identification of Organic Compounds. 7th ed. United States of America: John Wiley & Sons, Inc.; 2005.