Malaysian Journal of Chemistry, 2023, Vol. 25(1), 47-54

Quantification of Vitexin and Isovitexin in Seven Varieties of *Ficus deltoidea* in Peninsular Malaysia

Noraini Kasim[1,2], Adlin Afzan[2], Nurhuda Manshoor[3] and Nor Hadiani Ismail[1,3]*
[1] Faculty of Applied Science, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2]Atta-ur-Rahman Institute for Natural Product Discovery, Level 9, FF3, Universiti Teknologi MARA Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia
[3]Herbal Medicine Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, Setia Alam, 40170 Shah Alam, Malaysia
*Corresponding author (e-mail: norhadiani@uitm.edu.my)

Ficus deltoidea Jack (Moraceae), known as *mas cotek* in Malaysia is a popular herb of economic importance due to its various medicinal attributes. Seven varieties of *F. deltoidea* are available in Peninsular Malaysia, var. *angustifolia*, *bilobata*, *deltoidea*, *intermedia*, *kunstleri*, *motleyana*, and *trengganuensis*. In the Malaysian Herbal Monograph, vitexin and isovitexin are used as chemical markers for *F. deltoidea*. This paper reports the quantification of the markers in the leaf extracts of the seven varieties using two methods. The NMR box plot method provided approximate quantity, quickly revealing significant amounts of vitexin and isovitexin in var. *bilobata*. Meanwhile, var. *angustifolia* was dominated by isovitexin only. Var. *trengganuensis* and *intermedia* contained comparable amounts of both markers, *albeit* in significantly lower quantities. Through ultra-high performance liquid chromatography (UHPLC-DAD) analysis, vitexin content was determined to be highest in var. *bilobata* (27.21 µg/g of dry plant material), whilst isovitexin content was highest in var. *angustifolia* (12.97 µg/g of dry plant material). As in the NMR box plot approximation method, the UHPLC-DAD analysis also could not detect isovitexin in var. *deltoidea* and *kunstleri*. Differences in quantities of vitexin and isovitexin in the crude extracts could serve as a good indicator in distinguishing the varieties.

Keywords: *Ficus deltoidei*; chemical markers; isovitexin; vitexin; NMR box plot; UHPLC-DAD

Received: June 2022; Accepted: September 2022

Ficus deltoidea Jack (Moraceae) is a diverse species of subgenus Ficus. It is native and widely distributed throughout Malaysia, Thailand, Sumatra, Java, Kalimantan, Sulawesi, and the Moluccas [1]. This plant is a small shrub up to 3 m tall, sometimes occurring as an epiphyte [2]. It can be found in abundance along the beaches, in peat soils and in hilly forests up to 3000 m above sea level [3]. F. deltoidea is called 'Mas Cotek' in Malaysia due to the presence of golden dots on the upper surface of its lamina. The seven varieties of F. deltoidea; namely var. deltoidea, bilobata, angustifolia, intermedia, kunstleri, motleyana, and trengganuensis, found in the Peninsular Malaysia have been described by Kochummen [4].

F. deltoidea has been reported to possess various medicinal attributes, including aphrodisiac, antihypertension, anti-inflammatory, antidiabetic, and anticancer properties [5][6][7][8][9]. It is commonly consumed as tea, prepared from aqueous infusion of the dried leaves. Many commercial products in various forms such as drink, tea bag and tablets are in the market. Secondary metabolites of F. deltoidea include triterpenes, flavones glycosides, oligomer proanthocyanidins, and flavan-3-ol derivatives [10][11][12]. The flavonoids of F. deltoidea were reported to show various biological activities, including anti-oxidant and anti-inflammatory properties [11][13][14][15], cognitive effects [16][17], protective effect from cardiovascular disease [18], and anti-diabetic effects [19].

The Malaysian Herbal Monograph currently uses vitexin (apigenin-8-C-glucopyranoside) and iso-vitexin (apigenin-6-C- glucopyranoside) (Figure 1) as the chemical markers for *F. deltoidea* [20]. These metabolites are claimed to be responsible for the antidiabetic activity of the herb [21][22]. Both compounds have been reported to possess a wide range of pharmacological properties, including for treating cancer, cognitive deficits, depression, cardiac hypertrophy, hypertension, diabetes, obesity, infection, and metabolic disorders [23]. A bioassay-guided study targeting antidiabetic active compounds using α-glucosidase assay led to the isolation of vitexin and isovitexin from *F. deltoidea* [24]. Variability in the content of the marker compounds has been observed, however, the specification of the *F. deltoidea* varieties and the quantification of their chemical markers have not been critically undertaken.

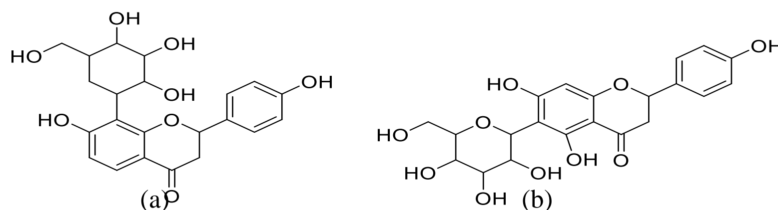

Figure 1. Structures of (a) vitexin and (b) isovitexin

This paper reports simultaneous quantification of vitexin and isovitexin in seven varieties of *F. deltoidea* available in Peninsular Malaysia (var. *trengganuensis*, *kunstleri*, *deltoidea*, *angustifolia*, *bilobata*, *intermedia*, and *motleyana*) using NMR and UHPLC methods. Samples for the analyses were comprehensively extracted using methanol. Convenient and reliable quantification methods for the marker compounds are important for quality control of *F. deltoidea* based herbal products.

EXPERIMENTAL

Chemicals

Vitexin and isovitexin primary analytical standards with 99.9% purity were purchased from ChromaDex (CA, USA). Ultrapure water was purified by Milli-Q system (MA, USA). Formic acid was obtained from Sigma Aldrich (MO, USA). Acetonitrile, methanol, potassium dihydrogen phosphate, and trimethylsilane propionic acid sodium salt (TSP) were supplied by Merck (Darmstadt, Germany). Deuterium oxide 99.9% (D_2O), methanol-d4 99.8% (CH_3OH-d_4) and sodium deuteroxide 99.9% (NaOD) were purchased from Armar Chemicals (Döttingen, Switzerland).

Materials

Reference specimens consisting of seven varieties of *F. deltoidea* (var. *angustifolia*, *bilobata*, *deltoidea*, *motleyana*, *intermedia*, *kunstleri*, *trengganuensis*) were obtained from the Germplasm Living Collection at the Gong Badak Campus of Universiti Sultan Zainal Abidin. The specimens were supplemented with complete phylogenetic analysis and voucher specimens [12]. After harvesting, all freshly collected leaves were wiped clean with methanol and placed in liquid nitrogen, before grinding using pestle and mortar. The resulting powdered plant materials were freeze-dried and kept at -80 °C until NMR and UHPLC-DAD analyses.

Methods

Nuclear Magnetic Resonance (NMR) Analysis

Powdered leaves (100 mg) were transferred into a micro-centrifuge tube and 1 mL of CH_3OH-d_4 was added. The mixture was vortexed for 2 min and sonicated for 20 min, followed by centrifugation at room temperature with a speed of 13,000 rpm for 5 min. The supernatant (500 µL) was transferred into a 2 mL micro-centrifuge tube. Then, 250 µL of KH_2PO_4 buffer (pH 6.0) containing 0.1% trimethylsilane propionic acid sodium salt (w/v) was added. The mixture was left for 30 min at 4°C, then centrifugation at 6000 rpm for 5 min. The supernatant (700 µL) was carefully transferred into a 5 mm NMR tube for analysis.

Nuclear Overhouser Effect Spectroscopy (NOESY) 1D-NMR was recorded at 300.1 K (26.8 °C) on a 600 MHz Bruker Ascend 600 spectrometer (Bruker, Massachusetts, USA), operating at the frequency of 600.30 MHz. CH_3OH-d_4 was used as internal lock. NOESY 1D-NMR spectra consisted of 8 scans requiring 4.94 sec acquisition time with the following parameters: 0.25 Hz/point, pulse width (PW) = 90° (10.8 µsec), and relaxation delay (RD) of 10 sec. A presaturation sequence was used to suppress the residual H_2O frequency during the recycle delay. The FIDs were Fourier transformed with line broadening (LB) = 0.3 Hz and the spectra were zerofilled to 32 K points [25].

The obtained NOESY 1D-NMR spectra were automatically converted to ASCII file using Chenomx software (version 5.1, Alberta, Canada). Spectral intensities were scaled to TSP and reduced to integrated regions of an equal width of 0.01 ppm bins, giving a total of 805 integrated regions per NMR spectrum. The averaged binned integral of the NOESY 1D-NMR data was subjected to multivariate data analysis using an online open access metabolomics software Metaboanalyst (version 4.0, Alberta, Canada) to generate boxplot for vitexin and isovitexin based on principal component analysis (PCA) loading plot. The relative amount of compounds was approximated based on the intensity of characteristic signals and translated into box-plot representation.

Ultra-High Performance Liquid Chromatography (UHPLC) Analysis

Dried leaves (1 g) of seven varieties of *F. deltoidea* were individually soaked in 10.0 mL of 100% methanol and sonicated for 30 minutes. The solvent was removed using a rotary evaporator and the resulting extracts were kept at 4°C until analysis. Sample clean-up was performed by dissolving the extracts in 2 mL of 85% aqueous methanol, followed

by sonication for 15 min and passed through solid phase extraction (SPE) cartridge. The SPE cartridges were activated by eluting with 6 mL of absolute methanol, followed by 6 mL of 85% aqueous methanol for conditioning. The samples were then introduced and eluted with 4 mL of 85% methanol and dried using Genevac EZ-2plus (Genevac LTD, Ipswich, UK). Prior to analysis, samples were reconstituted in 85% aqueous methanol at the concentration of 10 mg/mL.

All aqueous solutions were prepared using ultrapure water by Milli-Q system (MA, USA). The standard solutions were prepared by diluting the stock solutions with the solvent. The UHPLC analysis was performed on an Agilent 1290 Infinity II LC Series (Agilent Corporation, Santa Clara, USA) equipped with PDA detector, flexible pump and a vial-sampler manager. Chromatographic separation was achieved on an Agilent Zorbax RRHD Eclipse plus C_{18} chromatography column 2.1 mm x 150 mm, 1.8 µm. The column was maintained at 40°C in an oven and injection volume was 3 ul of 10.0 mg/mL extracts. The column was eluted with a solvent of 5–95% acetonitrile/water (0.1% v/v formic acid in both acetonitrile and water), gradient over 34 min at a flow rate of 0.3 mL/min.

The analyses were carried out in triplicates and the concentration of the marker compounds present in the samples were calculated by dividing the area under curve with the response factor. Results are presented as mean ± standard deviation. Statistical analyses were conducted using Microsoft Excel (version 2013, Washington, USA) and GraphPad Prism software (version 5.01, San-Diego, CA, USA). One-way ANOVA was used to analyze for significant differences. The difference between two groups is considered statistically significant when p-value is less than 0.05 ($p<0.05$).

UHPLC-DAD Method Validation

Aqueous methanol stock solutions containing 500 µg/mL of vitexin and isovitexin were prepared separately and diluted to 50, 100, 150, 200, and 250 µg/mL for high concentration and 2, 4, 6, 8, and 10 µg/mL for low concentration. The standards were analyzed in triplicate. The calibration curves were constructed by plotting the peak area of HPLC chromatograms versus the concentration. The linear regression equation of vitexin for high concentration standard at 50, 100, 150, 200, and 250 µg/mL is $y = 71.2x$ with regression coefficient of $r^2 = 0.999$ (Figure 2a). Whereas, for the low concentration of vitexin at 2, 4, 6, 8 and 10 µg/mL, the regression equation is $y = 75.82x$ with $r^2 = 0.997$ (Figure 2b).

For isovitexin, the linear regression equation for high concentration is $y = 73.86x$ with regression coefficient of $r^2 = 0.999$ (Figure 2c) and for low concentration, $y = 86.82x$ with $r^2 = 0.998$ (Figure 2d). The LOD was calculated based on the standard deviation of the response (Sy) of the curve and the slope of the calibration curve (S) at levels approximating the LOD according to the formula: LOD = 3.3(Sy/S). The standard deviation of the response was determined based on the standard deviation of y-intercepts of regression lines. The values of LOD for vitexin and isovitexin were 0.003 and 0.009 µg/mL, respectively.

The LOQ values were 0.009 and 0.028 µg/mL for vitexin and isovitexin, respectively. Relative standard deviation (RSD) indicates precision of measurement and is expressed in percentage, obtained by multiplying standard deviation by 100 and dividing this product by the mean. The RSD for vitexin was 0.07% and isovitexin was 0.25%.

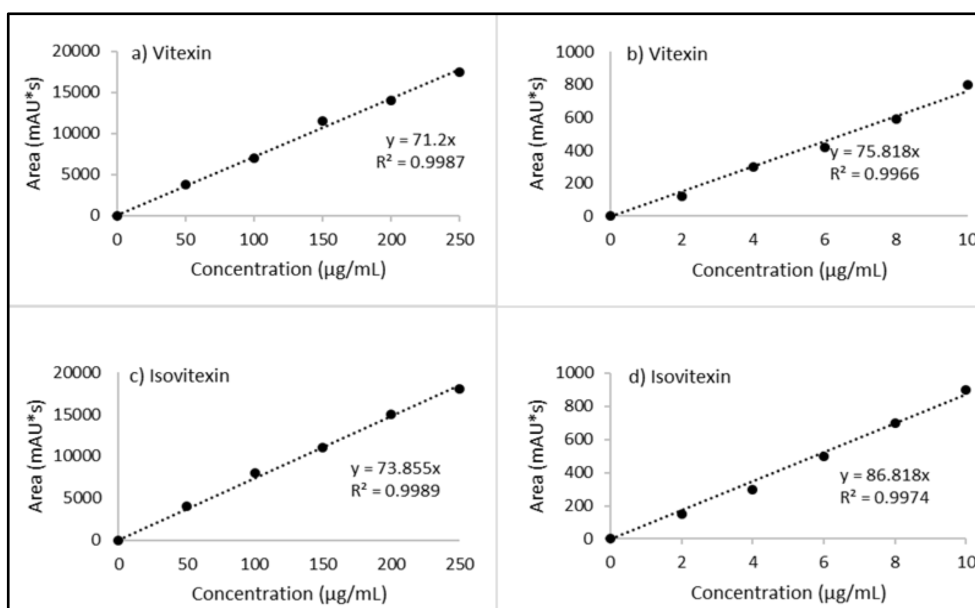

Figure 2. Calibration curves of: (a) vitexin in the range of 50.0 – 250.0 µg/mL; (b) vitexin in the range of 2.0-10.0 µg/mL; (c) isovitexin in the range of 50.0 – 250.0 µg/mL; and (d) isovitexin in the range of 2.0-10.0 µg/mL

Table 1. 1H chemical shifts of vitexin and isovitexin in *F. deltoidea* extract identified through 1D and 2D NMR spectra of the standards (in CD_3OD-KH_2PO_4 in D_2O, pH 6.0)

Marker	Chemical Shift (δ ppm)
Vitexin	7.99 (d), 7.02 (d), 6.66 (s), 6.32 (s), 5.03 (d)
Isovitexin	7.88 (d), 6.97 (d), 6.64 (s), 6.56 (s), 4.90 (d), 4.29 (t)

note: not including sugar signals

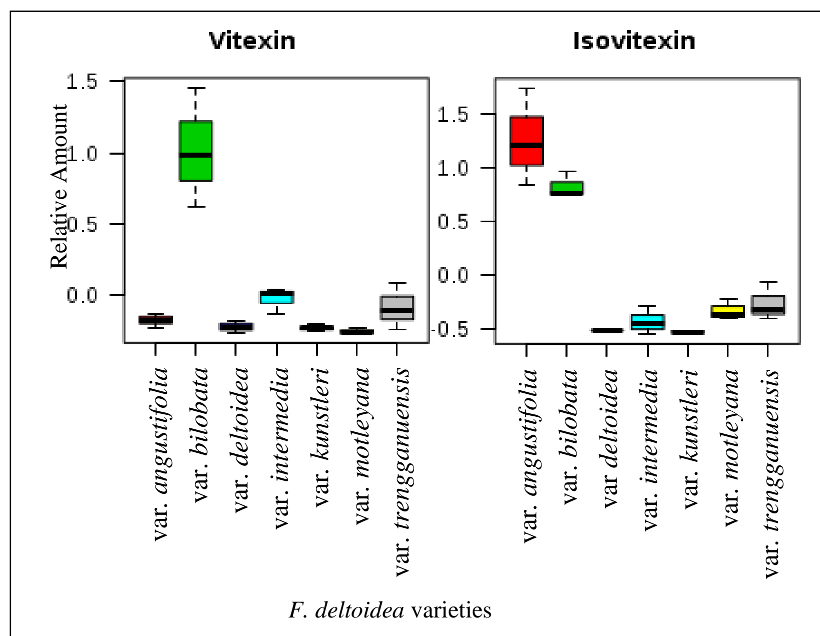

Figure 3. NMR box plots illustrating relative amounts of vitexin and isovitexin in seven *F. deltoidea* varieties

RESULTS AND DISCUSSION

Quantity approximation of vitexin and isovitexin in *F. deltoidea* varieties was performed by plotting their respective mean peak areas of the ^{1}H-NMR signals relative to the internal reference TSP after data binning. The characteristic NMR signals of vitexin and isovitexin, as shown in Table 1, were uploaded onto MetaboAnalyst 4.0 software for unsupervised principal clustering analysis (PCA). The markers were then relatively quantitated by the software and displayed in the form of box plots. The NMR box plots showed the variability in the vitexin and isovitexin contents among the seven *F. deltoidea* varieties (Fig.3). Significant amounts of both vitexin and isovitexin could readily be observed in var. *bilobata* through the NMR-based box plots, while var. *angustifolia* was dominated by isovitexin only. Var. *trengganuensis* and *intermedia* contained comparable amounts of both markers, but the quantities were significantly lower than var. *bilobata*. The amounts of vitexin and isovitexin could not be highlighted by the NMRbased box plots of other varieties due to their low concentrations. On the other hand, using this method, isovitexin was not detected in var. *deltoidea* and *kunstleri*. At first glance, these results provided rapid estimation of differences in quantities of the markers between varieties, and for more accurate quantification, the UHPLC analysis with high sensitivity was utilized.

The main challenge in the UHPLC quantification work was optimization of the chromatographic separation due to the complexity of the chemical profiles. Vitexin and isovitexin are isomeric compounds with the same sugar (glucose) attached at different positions of the apigenin aglycone. In vitexin, the glucose unit is attached to C-8, while in isovitexin it is attached to C-6. Both compounds elute very closely in HPLC separation. The best separation with resolution factor of 1.8 was achieved after careful adjustment of the acetonitrile/water solvent gradient. However, the retention time was still very close (vitexin, t_R 16.15 min; isovitexin, t_R 16.30 min). Because the signals were not fully resolved, the exponential skimming integration was applied for quantification using Agilent software analysis. The software was capable of selecting the unresolved peaks by integrating the peak located on the tailing edge of the other peaks. Figure 4 shows the UHPLC chromatograms of vitexin and isovitexin in the standard mixture and crude extracts of all the

seven varieties of *F. deltoidea*. The amounts of vitexin and isovitexin in dry leaves were calculated and expressed in µg/g plant material and percentage (Table 2). Analysis of samples of the seven varieties showed that the vitexin content in dry leaves varied between 0.00016 – 0.0072% with the highest quantity in var. *bilobata* and the lowest quantity in var. *kunstleri*. The highest content of isovitexin was observed in var. *angustifolia* (0.0013%), while for var. *kunstleri* and *deltoidea* was below the instrumental detection limit (LOD = 0.009 µg/mL). These results are in agreement with those reported by Abdullah *et al.* even though the previous work involved only three varieties, which were var. *trengganuensis*, *angustifolia* and *deltoidea*, performed on HPLC system [26].

From the results, NMRbased box plot and UHPLC methods are complementary to each other for the quantification of isovitexin and vitexin in crude extracts of F. deltoidea. Whilst the fast and simple NMR based box plot method was suitable for quick approximation, the tedious UHPLC method was necessary for absolute quantification. The NMRbased box plot method was convenient for screening purposes, however, lacked sensitivity to analyze compounds in very low concentrations. On the other hand, the UHPLC method was able to quantify the markers with the limit of quantification as low as 0.009 µg/ mL for vitexin, and 0.028 µg/mL for isovitexin. Previously, Azemin et al. reported High Performance Thin Layer Chromatography (HPTLC) method for analysis of vitexin and isovitexin in combination with multivariate analysis. In this work, F. deltoidea varieties were discriminated based on their vitexin and isovitexin contents [27]. However, the quantities of vitexin and isovitexin were not reported.

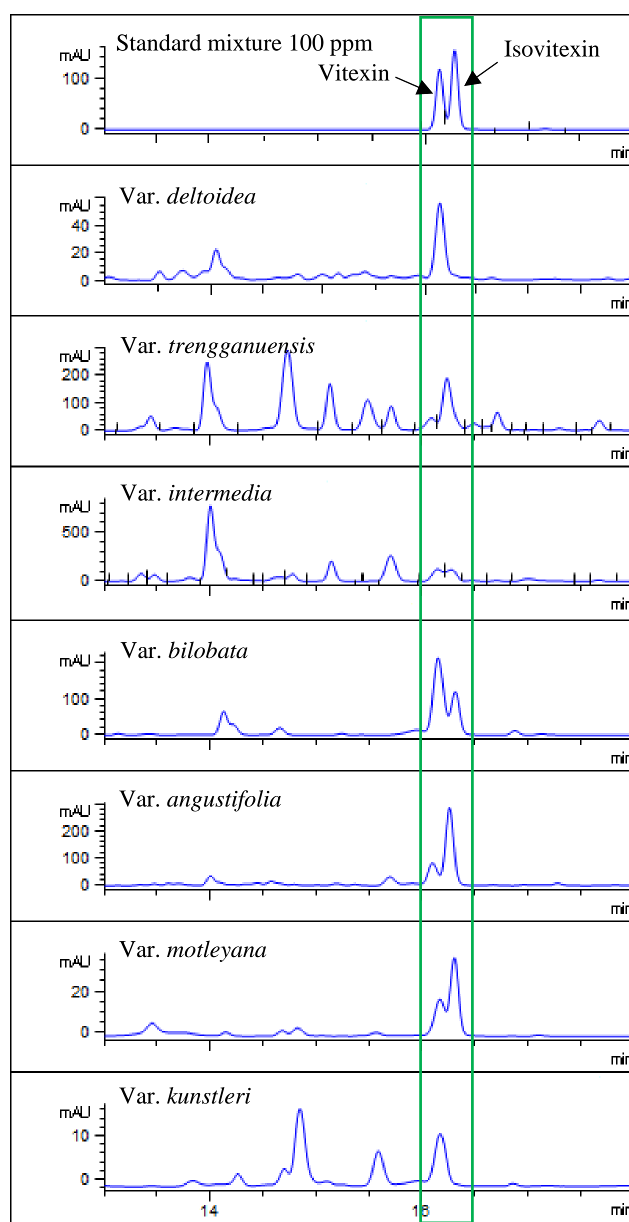

Figure 4. Chromatograms of vitexin and isovitexin of a standard mixture and *F. deltoidea* varieties

Table 2. Amount (µg/g) and % content of Vitexin and Isovitexin in Dry Plant Material

Varieties	Vitexin			Isovitexin		
	Concentration (µg/mL)	Amount (µg/g)	% Content	Concentration (µg/mL)	Amount (µg/g)	% Content
Var. *deltoidea*	29.61[a] ± 0.76	5.03	0.00050	nd	-	-
Var. *angustifolia*	12.07[b]± 0.55	1.98	0.00020	79.11[a]± 1.23	12.97	0.00130
Var. *kunstleri*	8.25[b]± 0.51	1.01	0.00010	nd	-	-
Var. *trengganuensis*	15.9[c]± 0.66	2.64	0.00026	71.51[b]± 1.46	11.87	0.00119
Var. *motleyana*	10.22[b]± 1.29	1.64	0.00016	15.25[c]± 1.31	2.44	0.00024
Var. *bilobata*	176.71[d]± 3.27	27.21	0.00272	66.94[b]± 1.73	10.31	0.00103
Var. *intermedia*	79.68[e]± 1.14	13.70	0.00137	61.12[d]± 1.39	10.51	0.00105
	LOD = 0.003 µg/mL LOQ = 0.009 µg/mL %RSD = 0.07			LOD = 0.009 µg/mL LOQ = 0.028 µg/mL %RSD = 0.25		

* ND = Not Detected
Values are expressed as mean ± standard deviation based on three replicates
[a,b,c,d,e,f,g,h] values with different letter show statistically significant difference ($p<0.05$).

Differences in chemical composition among varieties of the same species have been previously reported [28][29][30]. Differences in quantities of vitexin and isovitexin in the crude extracts could serve as a good discriminant factor to distinguish the varieties, which is crucial for correct selection of varieties for other studies and quality control.

CONCLUSION

This work successfully measured the relative and absolute quantities of vitexin and isovitexin using the NMR box plot and UHPLC analysis and illustrated the variability of the markers in seven varieties of *F. deltoidea* available in Peninsular Malaysia. The quantities of vitexin and isovitexin measured using the UHPLC method were more accurate and provided the absolute quantities of the markers, while the NMR box plot screening provided the approximate quantities of the metabolites. The NMR box plot can offer a simple, easy and quick relative quantification technique. It is an automated metabolite quantification based on variations in ^{1}H-NMR spectra prior to performing the complex UHPLC quantification. We recommend this combination of methods as a practical quality control tool, especially for analyzing raw *F. deltoidea* plant materials and herbal products.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Agriculture for supporting this work through research grant (100-RMI/MOA 16/6/2 (2/2013). The Ministry of Education and Ministry of Health are duly acknowledged for PhD scholarships granted to NK and AA, respectively. The research team gratefully acknowledges Prof A. Manaf Ali and Prof Nashriyah Mat for the plant samples.

REFERENCES

1. USDA (2007) ARS, National Genetic Resources Program, Germplasm Resources Information Network – (GRIN) Database. Beltsville, MD, USA: National Germplasm Resources Laboratory.

2. Corner, E. J. H. (1969) The complex of Ficus deltoidea; a recent invasion of the Sunda Shelf. *Philosophical Transactions of the Royal Society of London*, **256**, 281–317.

3. Musa, Y. (2005) Variability in morphology and agronomy of emas cotek accessions found in Kelantan and Terengganu. *Buletin Teknol Tanaman*, **2**, 35– 48.

4. Kochummen, K. M. (1978) Moraceae. In: Ng FSP (ed) Tree flora of Malaya (A manual of foresters). Malaysia, *Longman Malaysia Sdn. Bhd.*, 119 –168.

5. Hakiman, M. and Mahmood, M. (2009) Non enzymatic and enzymatic antioxidant activities in aqueous extract of different Ficus deltoidea accessions. *Journal of Medicinal Plants Research,* **3(3)**, 120–131.

6. Che Ahmad Tantowi, N. A., Hussin, P., Lau, S. F. & Mohamed, S. (2017) Mistletoe fig (Ficus deltoidea Jack) leaf extract prevented postmenopausal

osteoarthritis by attenuating inflammation and cartilage degradation in rat model. *Menopause*, **24(9)**, 1071–1080.

7. Adam, Z., Khamis, S., Ismail, A. & Hamid, M. (2010) Inhibitory properties of Ficus deltoidea on α-glucosidase activity. *Research Journal of Medicinal Plant*, **4(2)**, 61–75.

8. Nurdiana, S., Goh, Y. M., Hafandi, A., Dom, S. M., Syimal'ain, A. N., Syaffinaz, N. N. & Ebrahimi, M. (2018) Improvement of spatial learning and memory, cortical gyrification patterns and brain oxidative stress markers in diabetic rats treated with Ficus deltoidea leaf extract and vitexin. *Journal of Traditional and Complementary Medicine*, **8(1)**, 190–202.

9. Akhir, N. A. M., Chua, L. S., Majid, F. A. A. & Sarmidi, M. R. (2011) Cytotoxicity of aqueous and ethanolic extracts of Ficus deltoidea on human ovarian carcinoma cell line. *British Journal of Medicine and Medical Research*, **1(4)**, 397.

10. Lip, J. M., Hisham, D. N., Zaidi, J. A., Musa, Y., Ahmad, A. W., Normah, A. and Sharizan, A. (2009) Isolation and identification of moretenol from Ficus deltoidea leaves. *Journal of Tropical Agriculture and Food Science*, **37(2)**, 195–201.

11. Omar, M. H., Mullen, W. and Crozier, A. (2011) Identification of proanthocyanidin dimers and trimers, flavone C-glycosides, and antioxidants in Ficus deltoidea, a Malaysian herbal tea. *Journal of agricultural and food chemistry*, **59(4)**, 1363–1369.

12. Afzan, A., Kasim, N., Ismail, N. H., Azmi, N., Ali, A. M., Mat, N. and Wolfender, J. L. (2019) Differentiation of Ficus deltoidea varieties and chemical marker determination by UHPLC-TOFMS metabolomics for establishing quality control criteria of this popular Malaysian medicinal herb, *Metabolomics*, **15(3)**, 1–11.

13. Parhiz, H., Roohbakhsh, A., Soltani, F., Rezaee, R. and Iranshahi, M. (2015) Antioxidant and anti-inflammatory properties of the citrus flavonoids hesperidin and hesperetin: an updated review of their molecular mechanisms and experimental models. *Phytotherapy Research*, **29(3)**, 323–331.

14. Peluso, I., Miglio, C., Morabito, G., Ioannone, F. and Serafini, M. (2015) Flavonoids and immune function in human: a systematic review. *Critical reviews in food science and nutrition*, **55(3)**, 383–395.

15. Dueñas, M., Pérez-Alonso, J. J., Santos-Buelga, C. & Escribano-Bailón, T. (2008) Anthocyanin composition in fig (Ficus carica L.). *Journal of Food Composition and Analysis*, **21(2)**, 107–115.

16. Bell, L., Lamport, D. J., Butler, L. T. and Williams, C. M. (2015) A review of the cognitive effects observed in humans following acute supplementation with flavonoids, and their associated mechanisms of action. *Nutrients*, **7(12)**, 10290–10306.

17. Macready, A. L., Kennedy, O. B., Ellis, J. A., Williams, C. M., Spencer, J. P. and Butler, L. T. (2009) Flavonoids and cognitive function: a review of human randomized controlled trial studies and recommendations for future studies. *Genes & nutrition*, **4(4)**, 227.

18. Hodgson, J. M. (2006) Effects of tea and tea flavonoids on endothelial function and blood pressure: a brief review. *Clinical and experimental pharmacology and physiology*, **33(9)**, 838–841.

19. Vinayagam, R. & Xu, B. (2015) Antidiabetic properties of dietary flavonoids: a cellular mechanism review. *Nutrition & metabolism*, **12(1)**, 60.

20. Ismail, Z., Ismail, N. & Lassa, J. (1999) *Malaysian herbal monograph*, **1**.

21. Farsi, E., Ahmad, M., Hor, S. Y., Ahamed, M. B. K., Yam, M. F. and Asmawi, M. Z. (2014) Standardized extract of Ficus deltoidea stimulates insulin secretion and blocks hepatic glucose production by regulating the expression of glucose-metabolic genes in streptozitocin-induced diabetic rats. *BMC complementary and alternative medicine*, **14(1)**, 220.

22. Peng, X., Zheng, Z., Cheng, K. W., Shan, F., Ren, G. X., Chen, F. & Wang, M. (2008) Inhibitory effect of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation endproducts. *Food Chemistry*, **106(2)**, 475–481.

23. He, M., Min, J. W., Kong, W. L., He, X. H., Li, J. X. and Peng, B. W. (2016) A review on the pharmacological effects of vitexin and isovitexin. *Fitoterapia*, **115,** 74–85.

24. Choo, C. Y., Sulong, N. Y., Man, F. and Wong, T. W. (2012) Vitexin and isovitexin from the Leaves of Ficus deltoidea with in-vivo α-

glucosidase inhibition. *Journal of Ethnopharmacology*, **142(3)**, 776–781.

25. Bruker. Establishing the purity of nutraceuticals and energy drinks with NMR, Bruker application notes. https://www.bruker.com/es/resources/library/application-notes-mr/purity-of-nutraceuticals-and-energy-drinks-with-nmr.html.

26. Abdullah, Z., Hussain, K., Ismail, Z. & Ali, R. M. (2009) Anti-inflammatory activity of standardised extracts of leaves of three varieties of Ficus deltoidea. *International Journal of Pharmaceutical and Clinical Research*, **1(3)**, 100–105.

27. Azemin, A., Mohd, K. S., Juahir, H., Mat, N. & Ismail, Z. (2017) Discriminating factors for Ficus deltoidea Jack varieties by HPTLC coupled with Chemometrics. *Journal of Agrobiotechnology*, **8(2)**, 86–95.

28. Deng, Q., Penner, M. H. and Zhao, Y. (2011) Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. *Food Research International*, **44(9)**, 2712–2720.

29. Karimi, E. and Jaafar, H. Z. (2011) HPLC and GC-MS determination of bioactive compounds in microwave obtained extracts of three varieties of Labisia pumila Benth. *Molecules*, **16(8)**, 6791–6805.

30. Omer, E. A., Said-Al Ahl, H. A. H. and Hendawy, S. F. (2008) Production, chemical composition and volatile oil of different basil species/varieties cultivated under Egyptian soil salinity conditions. *Research Journal of Agriculture and Biological Sciences*, **4(4)**, 293–300.