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Chromatographic Fingerprint analysis for Determining the Presence of Vitexin and Isovitexin on Seven Varieties of Ficus Deltoidea

Zunoliza Abdullah*[1], Nor Azah Mohamad Ali[2], Mailina Jamil[2], Nashriyah Mat[3], Abdul Manaf Ali[3], Nurul Aina Awang[3], Wan Ainor Syahdah Wan Hassan[3], Ling Sui Kiong[1], Mohd Hafidz Hadi Abdullah[1], Nor Azlianie Ab'llah@Awang[1], Norfaizura Azmi[3]

[1]Phytochemistry Programme, Natural Products Division,
Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
[2] Herbal Product Development Programme, Natural Products Division,
Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
[3]School of Agriculture Science and Biotechnology, Faculty of Bioresource and Food Industry,
Universiti Sultan Zainal Abidin, Tembila Campus, Terengganu, Malaysia
*Corresponding author: zunoliza@frim.gov.my

Abstract

Ficus deltoidea Jack or locally known as mas cotek from the family of Moraceae has been acknowledged for its occurrence of several varieties based on the variations of leaf morphology, namely *F. deltoidea* var. *angustifolia* (Miq.) Corner, *F. deltoidea* var. *bilobata* Corner, *F. deltoidea* var. *kunstleri* (King) Corner, *F. deltoidea* var. *lutescens* (Desf.) Corner, *F. deltoidea* var. *trengganuensis* Corner, *F. deltoidea* var. *motleyana* and *F. deltoidea* var. *deltoidea* Jack. In this work, we report on the chemical profiles using chromatographic techniques on the seven varieties of *F. deltoidea* by analysing the presence of the bioactive compounds, vitexin and isovitexin and characterising the other major peaks in the chromatograms. Observation on the HPLC chromatogram showed the presence of both compounds in all samples except FDv. K (vitexin only) and FDv. I. Other peaks showed a characteristic of flavonoids based on the UV spectra pattern with UV maxima at 270 and 338 nm except for peaks 6, 7 and 8 with UV maxima at 294, 319 and 210, respectively. Both reference compounds were presence in varies concentration in all samples analysed.

Keywords: *Ficus deltoidea*; chromatographic analysis

INTRODUCTION

The usage of herbal based products has increased globally especially for complementary and alternative medicines. The expenditure on the traditional and herbal medicinal products has increased tremendously from $20 billion in 1997 to US$83 billion in 2008 [1-3]. Besides its lower price as compared to the modern drugs, the natural occurrence and the consumption from generation to generation are also the main reason as to why the herbal medicines and herbal based products have become more acceptable to the consumers [4]. Herbal medicines are usually herbal based products that contain the whole plants, parts of the plants or combination of the parts or plants either from raw material or in the extracted form. Some of them may contain sources of animal parts and/or minerals [5]. Various processing methods and combinations of the herbal materials have been used to maximize the therapeutic efficiency and minimize the toxicity of the herbal medicinal products [3, 6-9].

Despite the availability of many traditional/herbal medicinal products, the authenticity, quality, safety and efficacy studies on these herbal medicines remain a major thrust area in the research activities because the drug alteration and misidentification of herbal plants is still a major problem faced by the

herbal industry. Misidentification of herbal ingredients due to their different names resulted in consumption of wrong herbal medicines which may lead to unexpected effects. According to Kochummen [10] and Turner [11] as cited by A. Nasir et al [12] there are seven varieties of *Ficus deltoidea* (FD) has been recognised in Peninsular Malaysia namely FD var. *deltoidea*, var. *angustifolia*, var. *trengganuensis*, var. *bilobata*, var. *intermedia*, var. *kunsleri* and var. *motleyana*. Despite of myriad herbal products containing FD as one of the ingredients, the identity of varieties that has been used in the preparation has always been query. The correct identification of varieties is important in maintaining the quality of products. Therefore, the present study has been conducted to develop an analytical technique that can be used in the identification and authentication of FD varieties.

The identification and authentication of the herbal plants are usually carried out by studying the botanical anatomy and phytochemical profiles. Analytically, the secondary metabolites such as carotenoids/chlorophylls/phenolic acids/flavonoids/alkaloids/antocyanins/terpenoids and phytosterols have been used as markers or reference compounds in the identification and authentication. These phytochemicals have played important roles in either the attraction or defence mechanism. Polyphenols and its derivatives are the most important secondary metabolites found in medicinal plants which have shown wide ranges of beneficial effects such as antibacterial, anticarcinogenic, anti-inflammatory, antiviral, antiallergic, estrogenic and immune-stimulating properties [13].

The fingerprint analysis that evaluates the integrative and holistic properties of herbal medicines has been acceptable internationally as one of the efficient methods in quality control of herbal medicines [14]. The Food and Drug Administration, FDA [15] and European Medicines Agency, EMEA [16] have denoted the application of appropriate fingerprint chromatogram in determining the consistency of botanical drugs. Several analytical techniques have been applied in the determination of the quality of herbal plants. One of the techniques, namely chromatographic fingerprinting is the most powerful approach [4,17] in the development of phytochemical profile that will provide information on the appearance of peaks that feature the similarity or dissimilarity between various samples of herbal plants that are used in the preparation of herbal medicines. Chromatographic fingerprinting can be carried out using techniques such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and other hyphenated techniques.

Ficus deltoidea or locally known as mas cotek from the family of Moraceae is one of the most popular herbal plants that has been used among the Malays. This plant is traditionally being used for treating wounds, rheumatism and sores. A decoction of the leaves is also used by women after giving birth to improve blood circulation and regain body strength. Some ethnic groups in East Malaysia consume the plant as herbal tea for anti-aging and young appearance. Several biological studies have been reported on this plant which included antioxidants [18-22], antihypertensive [23-24], anti-diabetic [25-30], anti-inflammatory [20,31], antimelanogenic and antiphoto-aging effect [32-33], antiulcerogenic [34], wound healing [35], antibacterial [36-37] and anticancer [38]. Acute and subchronic toxicity study by Farsi et al [39] showed that there were no significant adverse effects of toxicity symptom on the Sprague Dawley rats.

Research also showed that *F. deltoidea* possesses five active components which are required by the human body, namely flavanoids, tannins, triterpenoids, proanthocyanins and phenols [20, 32]. Omar et al [22] reported the presence of antioxidant flavonoids including flavan-3-ol monomers namely; catechin, epicatechin, gallocatechin and epigallocatechin in the aqueous extracts. A few chemical compounds have been found present in the leaves of *F. deltoidea* which included prangenin, 3,5-di-tertbutylphenol, methyl 3-(3,5-ditertbutyl-4-hydroxyphenyl, vitexin, isovitexin and moretenol [20, 25, 40]. Two of the compounds, namely vitexin and isovitexin have been reported to play a role as an antidiabetic agent through in-vivo α-glucosidase inhibition [41]. Both of these compounds have been used as the reference marker for the chemical profiling of three varieties of *Ficus deltoidea* leaves or its extracts [20]. There were several articles reporting on the pharmacological activities of these compounds such as antihypertensive, anti-inflammatory, antispasmodic, antimicrobial and antioxidant [42-44].

In this study we have utilized both of the compounds; vitexin and isovitexin as reference markers for chromatographic fingerprint analysis on seven varieties of *Ficus deltoidea* that are available in the Peninsular of Malaysia. The analysis also included the characterization of the other major peaks in the chromatograms.

EXPERIMENTAL

Plant materials and chemicals

The leaves of all the seven varieties of *Ficus deltoidea* were harvested from the germplasm plot at Universiti Sultan Zainal Abidin (Unisza) located at Besut, Terengganu. The samples are listed in Table 1. Vitexin and isovitexin were used as the reference compounds. HPLC grade acetonitrile and water were used for the HPLC analysis.

Table 1 List of varieties of *Ficus deltoidea* leaves

No.	Samples	Code	Herbarium No.
1	*Ficus deltoidea* var *motleyana*	FDv. M	00313
2	*Ficus deltoidea* var *bilobata*	FDv. B	00057
3	*Ficus deltoidea* var *intermedia*	FDv. I	00308
4	*Ficus deltoidea* var *kunstleri*	FDv. K	00312
5	*Ficus deltoidea* var *deltoidea*	FDv. D	00367
6	*Ficus deltoidea* var *terengganuensis*	FDv. T	00300
7	*Ficus deltoidea* var *angustifolia*	FDv. A	00331

Preparation of samples

Dried and ground samples were extracted using methanol at room temperature with a ratio of 1 g: 10 mL. The extracts were filtered and then dried in vacuo at 40°C. 40 mg of each sample was taken and re-dissolved in 1 mL of methanol (HPLC grade) for the analysis. Formic acid was of analytical grade.

Preparation of standard and extract solution

Standard stock solution of vitexin and isovitexin were prepared at a concentration of 1 mg/mL in methanol. The solution was filtered through 0.45 µm PTFE membrane filter.

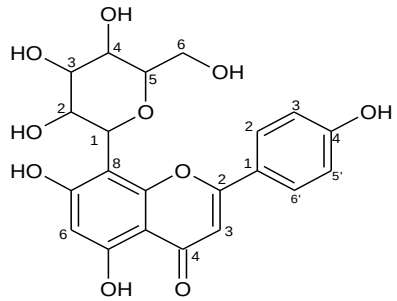
Instrumentation and chromatographic conditions

HPLC analysis was performed on WATERS 600E pump attached with 2996 photodiode array detector and data were analysed using Empower software. The analysis was carried out on a reversed phase column, Purospher® STAR (250 x 4.6 mm, 5 µm). The solvent system consisted of 0.1 % formic acid in water (A) and acetonitrile (B) with a linear gradient elution developed over 45 minutes running time. The flow rate was set at 1 mL/min and the injection volume was 10 uL.

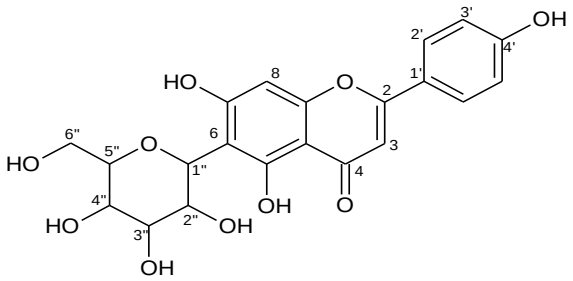
RESULTS AND DISCUSSION

Studies conducted by several researchers [20, 22, 25, 40] have reported the presence of a few types of chemical components in *Ficus deltoidea* which included catechin, epicatechin, gallocatechin and epigallocatechin, prangenin, 3,5-di-tertbutylphenol, methyl 3-(3,5-ditertbutyl-4-hydroxyphenyl, vitexin, isovitexin and moretenol. However, in the present study, only vitexin and isovitexin were used as the

reference markers due to its role as antidiabetic agent in *in vivo* α-glucosidase inhibition as reported by Choo et al [41]. The potential inhibition of these compounds on α-glucosidase activity also been reported by Yao et al [45]. The chromatographic profile has been optimized using different types of columns with different combinations of mobile phase. Satisfactory chromatographic profile was obtained after using the reversed phase column, Purospher® STAR with gradient elution for 45 min. Both compounds showed maximum absorptions (λ_{max}) at 270 nm and 330 nm. The chromatograms and UV spectra of vitexin (**1**) and isovitexin (**2**) extracted at 270 nm are shown in Figure 1. They were eluted at retention times 26.136 min and 27.227 min, respectively. Figure 2 displayed the chromatograms and UV spectra of the major peaks observed in all the seven varieties of *Ficus deltoidea* leaves. The presence of both compounds; vitexin and isovitexin were observed and labelled as peak 9 and 10, respectively by the comparison of their retention times and UV spectra. Figure 2 also showed the presence of other peaks which exhibited similar UV spectral characteristics of vitexin and isovitexin, an apigenin flavone glucoside. Besides that, the HPLC chromatogram also exhibited three other peaks with different pattern from the vitexin and isovitexin labelled as peak 6, 7 and 8 with UV maxima at 294 nm, 319 nm and 310 nm, respectively. As observed in the chromatograms, both vitexin and isovitexin can be found in all varieties of *Ficus deltoidea* (*Ficus deltoidea* var *motleyana*, *Ficus deltoidea* var *bilobata*, *Ficus deltoidea* var *deltoidea*, *Ficus deltoidea* var *terengganuensis* and *Ficus deltoidea* var *angustifloia*) except in *Ficus deltoidea* var *intermedia* and *Ficus deltoidea* var *kunstleri*. Table 2 exhibited the concentration of vitexin and isovitexin calculated in five varieties of *Ficus deltoidea*. As calculated from the linear plot of vitexin (R^2=0.9999) and isovitexin (R^2=0.9996), FDv. B showed the highest concentration of vitexin with 298.28 ± 3.97 µg/mL, while the FDv. A showed the highest concentration of isovitexin with 184.50 ± 0.35 µg/mL.



Vitexin (**1**)



Isovitexin (**2**)

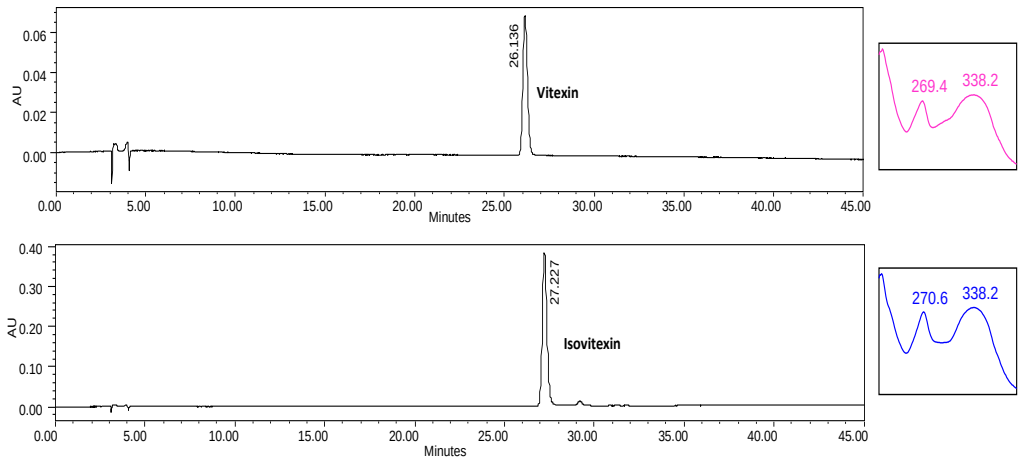


Figure 1: HPLC chromatogram of vitexin and isovitexin at 270 nm

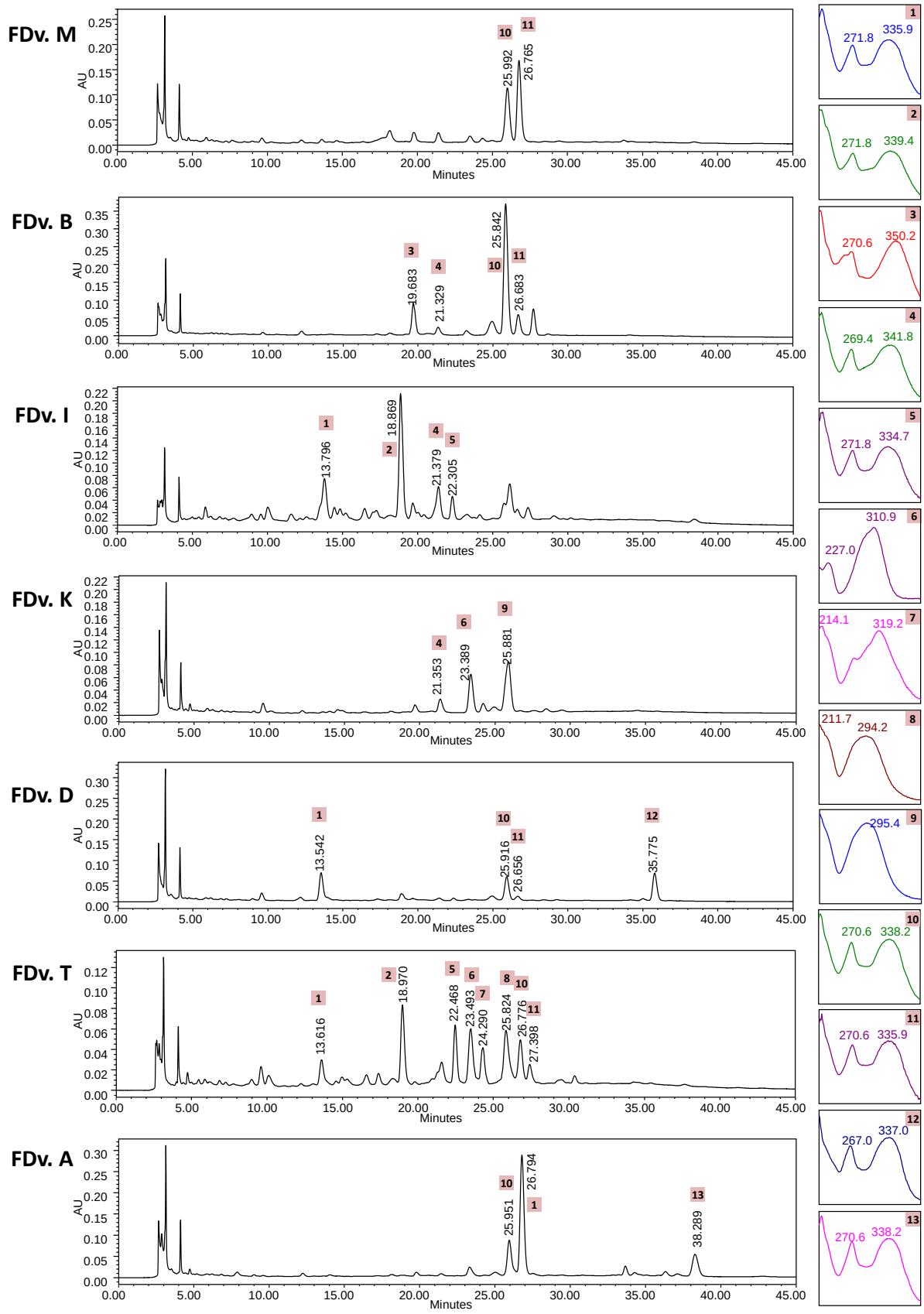


Figure 2 HPLC chromatogram and UV spectra of seven varieties of *Ficus deltoidea* at 270 nm

Table 2 Retention time and calculated concentration of vitexin and isovitexin in five varieties of *Ficus deltoidea*

Sample code	Vitexin		Isovitexin	
	RT (min ± SD)	Concentration ± SD (µg/mL)	RT (min ± SD)	Cconcentration ± SD (µg/mL)
FDv.M	25.99 ± 0.02	94.68 ± 0.22	26.78 ± 0.03	106.32 ± 0.87
FDv.B	25.82 ± 0.03	298.28 ± 3.97	26.66 ± 0.04	33.54 ± 0.97
FDv.D	25.96 ± 0.03	46.29 ± 0.70	26.69 ± 0.03	3.26 ± 0.42
FDv.T	26.78 ± 0.02	28.77 ± 0.62	27.40 ± 0.02	7.12 ± 0.43
FDv.A	25.94 ± 0.04	64.01 ± 1.52	26.77 ± 0.04	184.50 ± 0.35

Values are means ± standard deviation

CONCLUSION

A HPLC method for chromatographic fingerprint analysis using vitexin and isovitexin as the reference compounds for *Ficus deltoidea* has been successfully developed. The developed method also showed the presence of other compounds that showed similar UV spectra with the reference compounds. The chromatograms also exhibited the presence of peaks belonging to other types of compounds such as the phenolics. These chromatographic fingerprints and method developed could be employed as one of the analytical methods in the quality control and authentication of raw materials of *Ficus deltoidea*. The concentration of vitexin and isovitexin varies in all five varieties that showed the presence of both reference compounds. The highest concentration of vitexin and isovitexin was calculated in *Ficus deltoidea* var. *bilobata* and *Ficus deltoidea* var. *angustifolia*, respectively.

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