

Comparison of Chemical Profile and Biological Activities of Different Plant Parts of *Ficus deltoidea* Jack var. *Trengganuensis*

Mohd K S^{1*}, Azemin A¹, Rosli A S¹, Zakaria A J², Ismail Z³

¹School of Agriculture Sciences and Biotechnology, Faculty of Bioresources and Food Industry, Malaysia

²Research Institute for Agricultural Production and Food Innovation (AGROPOLIS), Universiti Sultan Zainal Abidin, Tembilika Campus, 22200 Besut, Terengganu, Malaysia

³School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia

Available Online: 15th March, 2015

ABSTRACT

Ficus deltoidea var. *Trengganuensis* (FDT) is one of the variety of a highly potential herbs, *Ficus deltoidea* Jack. This herb has pharmacologically been shown to possess numerous biological activities. Commonly, only leaves have been used medicinally. In order to assess whether other part such as fig and stem can also be used for medicinal purposes, chemical profile and biological activities were compared. Samples that consist of leaves, figs and stems were collected from four locations in Trengganu state, east coast of Peninsular Malaysia. Thin layer chromatography (TLC) and Fourier transform infrared (FTIR) were used to assess the chemical profile. Two compounds, vitexin and isovitexin were used as authentication marker of FDT. A good separation of the chemical components and marker in all sample with distinct spots observed on fig TLC chromatogram at the solvent system of ethyl acetate: formic acid (0.1%): methanol (4:6:2 v/v/v). FTIR fingerprints also exhibit different profile for fig, stem and leaf extract. Principle component analysis on FTIR data revealed that figs from four locations clustered into one group in positive PC2 quadrant suggest that fig has specific constituents, which discriminate them from leaf and stem. Biological activities were assessed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) inhibition and α -glucosidase inhibition assays. Comparison between plant parts discovered that figs from all locations have the highest inhibition on both activities compared to leaves and stems. Finding from this study revealed that figs of FDT could also be consumed for therapeutic purposes in addition to the leaves.

Keywords: *Ficus deltoidea* Jack, FTIR fingerprint, vitexin, isovitexin, DPPH

INTRODUCTION

Ficus deltoidea Jack (FD) or vernacularly known as Mas cotek, Serapat Angin, Sempit-sempit and Angoluran bang is a highly potential herb that have been reported used to treat diabetic, high blood pressure, fever, cold, headache, tooth pain, migraine, gout and high cholesterol [1-7]. FD also has strong anti-oxidant activity which good as anti-aging [8-9].

It is believed that root, bark, leaf and fig (fruit) of *Ficus deltoidea* have medicinal properties [12]. However, to date there are still lack of study on chemical property and biological activity of other plant parts other than the leaves. Though, there are reports on biological activities on fig. The figs of *Ficus deltoidea* var. *angustifolia* have shown to possess good source of antioxidant [13] and its aqueous extract shows good anti-inflammatory activity [14]. FD figs have also been traditionally chewed to treat tooth pain, cold and headache [15]. Studies also suggest that figs and leaves are comparable in their ability to treat diseases. Misbah et al. [16] reported that figs extracts of *Ficus deltoidea* var. *angustifolia* and var. *kunstleri* showed effective anti-diabetic properties. They also stated that antioxidant activity of the figs might be due to the phenolic content as it was correlated positively with each other.

Aqueous extract of both leaves and figs also significantly reduced the external glucose load at 50mg/kg dosage [17]. In another study figs have shown to have higher phenolic content than leaves and exhibited comparable results for antioxidant activities and neuroprotective effect [18].

There are at least seven varieties of *Ficus deltoidea* identified in Malaysian Peninsular. One variety in particular can be found in heath forest and its surrounding area identified as *Ficus deltoidea* var. *trengganuensis* (FDT). According to Corner [10], FDT is distributed around Terengganu and east coast of Pahang, east coast of Peninsular Malaysia. It is an epiphytic in lowland forest, in coastal shrub and in *Leptospermum* forest at 1300 m alt. The twigs are 2 mm thick and 2.3 x 1.8-5.5 cm, elliptic to rounded-obovate lamina or somewhat bilobed at the apex with fig-body 9-12 mm wide in rose-red to purple- black color [10]. Recent study has reported that FDT have similar chemical component to other varieties [11] even though it grows on considerable 'infertile' Beach Ridges Interspersed with Swales (BRIS) soil. BRIS soil is an acidic, sandy soil, high percentage of silica and poor in its nutrients found in a tropical moist forest or heath forest. Taking into consideration of its availability and easily collected from the wild or through cultivation as compared

to other varieties, it is worth to evaluate chemical and biological properties of different parts of FDT. In order to assess whether other parts such as fig and stem can also be used as medicinal purposes, chemical profile and biological activities were compared. Thin layer chromatography and Fourier transform infrared were utilized to establish the fingerprint of fig, stem and leaf of FDT collected from four localities. For authentication, vitexin and isovitexin were used as markers. In order to evaluate the chemical fingerprint of different parts from different localities, multivariate analysis was employed to get the insight of the possible discrimination between samples. For the comparison of biological activities, the inhibition of scavenging activity by DPPH and the inhibition of alpha glucosidase enzyme were examined. We hypothesized that fig, stem and leaf possess different chemical profile, which eventually determine the capacity of the biological activity.

MATERIALS AND METHODS

Sample preparation

The plant samples of *FD* var. *trengganuensis* were collected from four localities around Terengganu namely Tok Jembal (05° 23.644N, 103° 05.656E), Gong Datuk (05° 24.395N, 103° 04.677E), Mengabang Telung (05° 43.760N, 103° 04.970E) and Rhu Tapai (05° 51.520N, 102° 97.110E). The plant samples were separated into leaves, stems and figs. All samples were dried in conventional oven at 45°C then ground into powder. Accurately weighted 50 g of leaf, stem and fig powder were extracted with methanol for three days and the filtrates were concentrated under pressure at 45°C. The crude extracts were kept in -20°C prior analysis.

Thin Layer Chromatography

Thin layer chromatography was performed to analyze the chemical composition of FDT samples. The protocol is in accordance to [11] with slight modification. Accurately 2 µL of each standard and samples solutions were spotted on TLC aluminum sheet pre-coated silica gel 60 F₂₅₄ (Merck, Germany). The plate was developed in the mobile phase, ethyl acetate: formic acid (0.1%): methanol (4:6:2 v/v/v). After development, the plate was dried in the air and scanned at 265 nm, 366 nm and derivatized with vanillin reagent.

Fourier Transform Infrared analysis

Samples were scanned directly by using attenuated total reflectance-fourier transform infrared (ATR-FTIR). The FTIR spectra were obtained and recorded at the range of 4000-600 cm⁻¹ using a Perkin Elmer Spectrum 400 Infrared spectroscope coupled with air-cooled deuterated triglycine sulphate (DTGS). The infrared measurements were made at a resolution of 4 cm⁻¹, and 16 interferograms were co-added before the fourier transformation.

Principal Component Analysis

Baseline correction was performed using the *Spectrum* (PerkinElmer, Inc.) software in order to minimise the differences between the FTIR spectra due to baseline shifts. The FTIR spectra were exported as *Spectrum.SP* file and then were imported into multivariate statistical software program, *The Unscrambler X* (CAMO,

Trondheim, Norway) and were normalized. The principal component analysis (PCA) was performed using *The Unscrambler X*. PCA was used to discriminate the plant parts of FDT from four localities.

Biological Assay

DPPH antioxidant assay

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay for antioxidant activity was carried out followed as Dzolin et al. [18] with some modification. The stock solutions of samples were prepared in 1 mg/ml in methanol and were made serial dilutions for different concentration in 96-well microtiter plate. Quercetin was used as positive control. Then, 200 µl of DPPH in methanol solution (6 x 10⁻⁵ M) were added into each well. The plate was shook gently and was incubated in dark room for 30 minutes at room temperature. The reaction was measured by absorbance at 517 nm. The percentage of inhibition was calculated using the following formula:

Percentage of inhibition:

$$[1-(A_{517nm, sample} - A_{517nm, control}) \times 100]$$

Alpha glucosidase inhibition assay

The alpha glucosidase method was performed according to Mayur et al. [19] with modification. The stock solution of samples (1 mg/ml) in DMSO, 10% DMSO, phosphate buffer (pH 7), sodium carbonate, alpha glucosidase (enzyme) and 4-nitrophenyl alpha-D-glucopyranoside (substrate) were prepared. Ten microliters of samples were mixed with the 10% DMSO in 96-well microtiter plate and were made serial dilution for different concentrations. Fifty microliters of 0.1 M phosphate buffer and 25 µl of alpha-glucosidase solution were added into well plate and incubated for 15 minutes at 37°C. Twenty five microliters of 0.5 mM 4-nitrophenyl alpha-D-glucopyranoside substrate was added to complete the reaction and incubated for another 30 minutes at 37°C. The reaction was terminated by adding 100 µl of 0.2 M sodium carbonate solution. Acarbose was used as positive control. The absorbance was measured at 410 nm. The percentage of inhibition was calculated using the following formula:

Percentage of inhibition:

$$[(Control\ abs - sample\ abs)/control\ abs] \times 100$$

RESULTS AND DISCUSSION

Chemical Profile

The separation of chemical compositions of leaves, stems and figs of FDT from four localities were obtained based on the separation of its marker compounds namely vitexin and isovitexin. Figure 1 shows TLC chromatograms that were visualized under UV light, (a) 265 nm (b) 366 nm and (c) after derivatization with vanillin reagent. In general, TLC profile indicates variability in chemical composition and fig extracts shown to have the most chemical constituents compared to leaf and stem extracts at chosen solvent system. The excitation of compounds under 366 nm of fig extracts from four localities reveals a distinct chemical compounds which present as blue and green fluorescence bands. Those bands do not seen in leaf and stem samples (Fig. 1(b)). The chromatogram of vanillin-

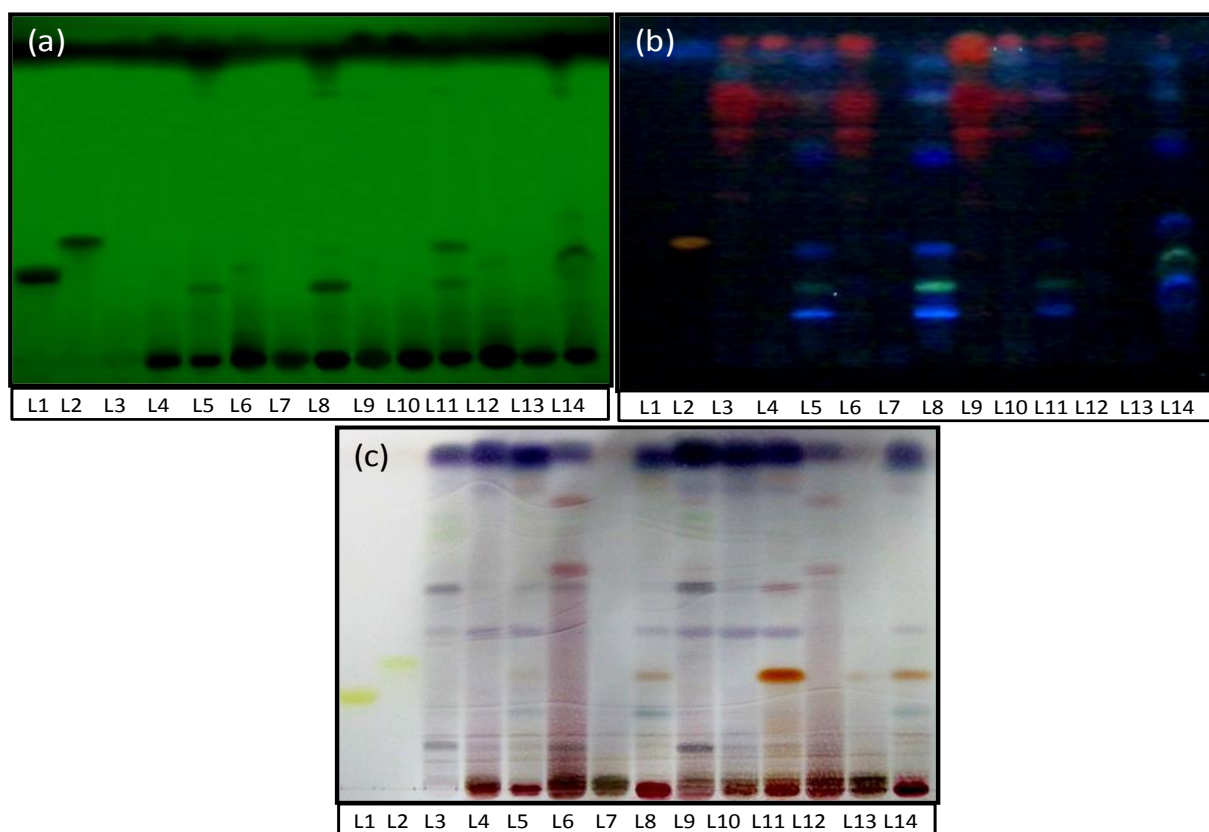


Figure 1: Methanol extract of leaf, stem and fig of FD var. *trengganuensis* from four localities and marker compounds (isovitexin and vitexin). TLC chromatograms were visualized (a) 265 nm (b) 366 nm (c) after derivatization with Vanillin reagent. L1: Isovitexin; L2: Vitexin; L3: Leaf (Tok Jembal); L4: Stem (Tok Jembal); L5: Fig (Tok Jembal); L6: Leaf (Gong Datuk); L7: Stem (Gong Datuk); L8: Fig (Gong Datuk); L9: Leaf (Mengabang Telung); L10: Stem (Mengabang Telung); L11: Fig (Mengabang Telung); L12: Leaf (Rhu Tapai); L13: Stem (Rhu Tapai); L14: Fig (Rhu Tapai).

derivatized plate showed more compounds occur in fig extract compared to that of leaf and stem in all localities (Fig. 1(c)). Those fluorescence bands indicate the presence of flavonoids compounds as previous reported^[20].

Typical FTIR spectra for all samples are shown in Figure 2. The spectra of leaf and stem samples are similar but different from that of fig in all localities. Generally, there were four main differences between the spectra of leaf, stem and fig. Peak approximately at 1375 cm^{-1} represented as symmetrical bending vibration of C-H bonds ($\delta_s\text{ CH}_3$) occurs strongly in leaf and stem spectra but weak in fig. Meanwhile, OH stretching and bending vibration (primary and secondary, OH in plane bend) attributable at bands $1350\text{-}1260\text{ cm}^{-1}$ was present only in fig spectra. The frequencies ranged between $922\text{-}919\text{ cm}^{-1}$ and $700\text{-}610\text{ cm}^{-1}$ only exist in stem and leaf which assigned as isopropyl group (C-H bending vibrational Gem-dimethyl group) and C-H bending vibration of alkynes, respectively. The discrimination between samples was further analyzed by multivariate analysis on FTIR data. Figure 3 shows PCA scores plot of three plant parts of FDT from four localities in 2D and 3D view. The scores plot shows that all samples are significantly discriminate from each other. Fig extract from different localities clustered together in the same quadrant, positive PC-2 and negative PC-1 indicating the

occurrence of similar chemical constituents among figs samples but differ from leaf and stem.

Biological activities

An antioxidant is defined as any substance that significantly delays or inhibits oxidation of a substance when present at low concentrations compared to that of an oxidisable substrate^[21]. DPPH antioxidant activity was measured by the donation of hydrogen atom from antioxidant compounds (in sample extracts) to DPPH, a stable free radical causing radicals scavenged and the absorbance is reduced as DPPH shows the maximum absorbance at 517 nm ^[21,22]. The scavenging of free radicals turns the colour of solution from purple to yellow that indicate DPPH is reduced to diphenylpicrylhydrazine. The results of DPPH antioxidant activity of plant parts from four localities were shown in Figure 4. Fig samples from Gong Datuk, Mengabang Telung and Rhu Tapai possess radical scavenging inhibition of 82.24%, 82.22% and 75.77%, respectively. Highest activity shown by figs, followed by leaves (81.31%, 71.20% and 71.17%, respectively) and stems (50.13%, 70.91% and 51.14%, respectively). In contrast, sample from Tok Jembal showed different result where the highest activity occurred in stem (80.60%), which was slightly higher than fig (80.35%) while leaf (53.52%) had the least activity. The 50% inhibition of extracts for DPPH antioxidant activity from

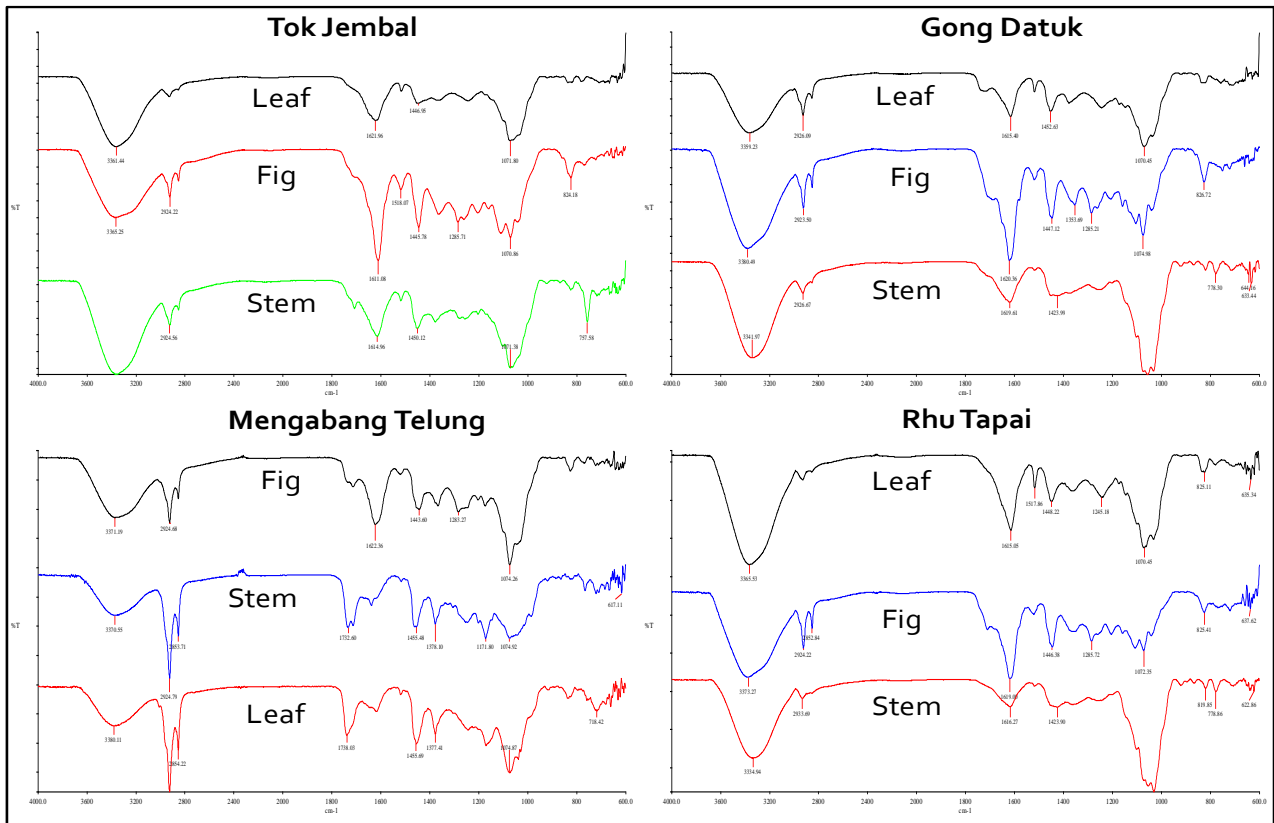


Figure 2: Typical FTIR spectra of plant parts of FDT from four localities.

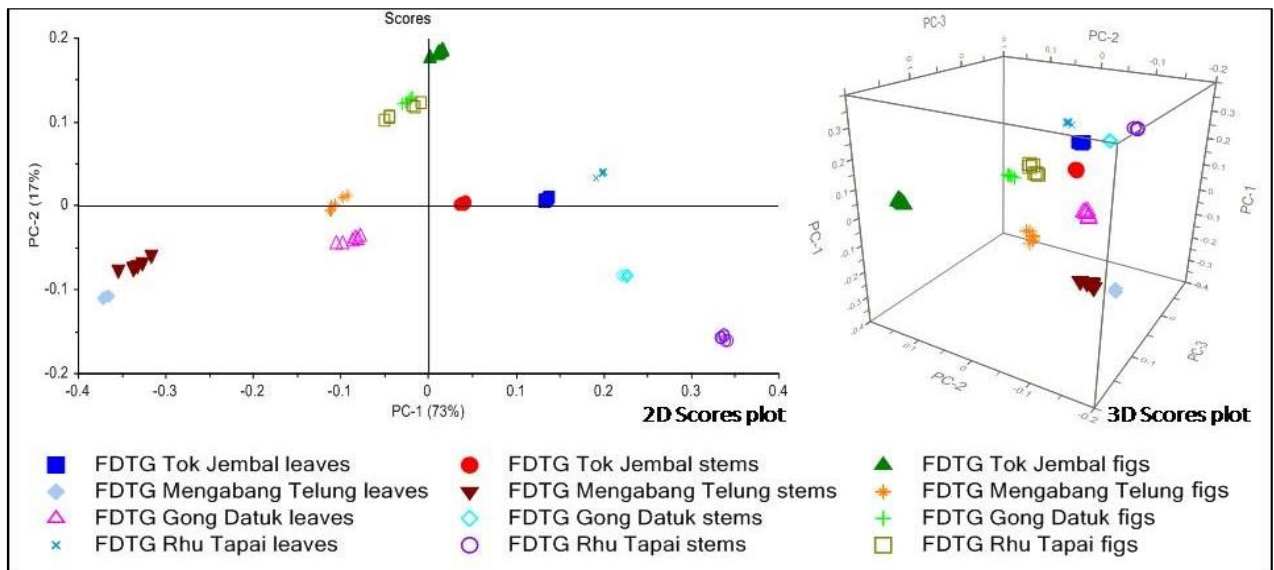


Figure 3: PCA scores plot of three part of FDT from four localities in 2D (left) and 3D (right) view. FDTG: *Ficus deltoidea* var. *trengganuensis*

four localities in Figure 5 shows that figs extract scavenged free radicals at lowest concentration. Thus, we reasoned that figs had shown the highest antioxidant capacity among FDT plant parts of all four localities. In the same vein, cluster analysis by PCA have shown that samples that exhibit higher antioxidant activity were clustered together at the top quadrant of PC2 and samples that clustered at the bottom of PC2 quadrant have moderate or weak inhibition capacity. We presumed that high antioxidant activity of fig

extracts could be contributes by flavonoids compounds as shown by their TLC profile. Flavonoids and phenolic acid are known for their ability to act as antioxidants and have shown present naturally in vegetables, fruits and seeds [21]. Alpha-glucosidase is a membrane-bound enzyme located at the epithelium of small intestine, where it break down by chemical reaction with water (hydrolyzes) at the final step in digestion of carbohydrates [23]. The inhibition of alpha glucosidase enzymes can significantly decrease

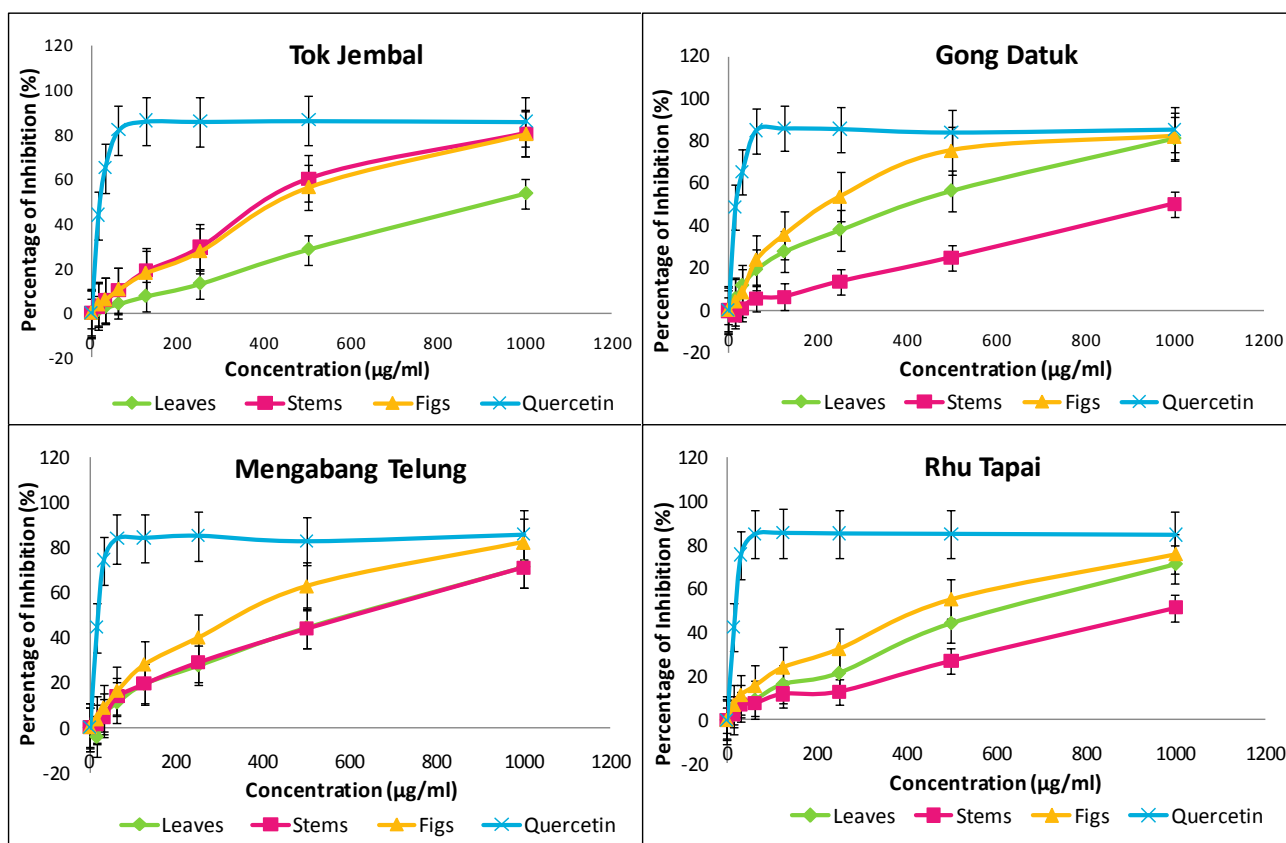


Figure 4. DPPH antioxidant activity of three plant part of FDT from four localities around Terengganu areas.

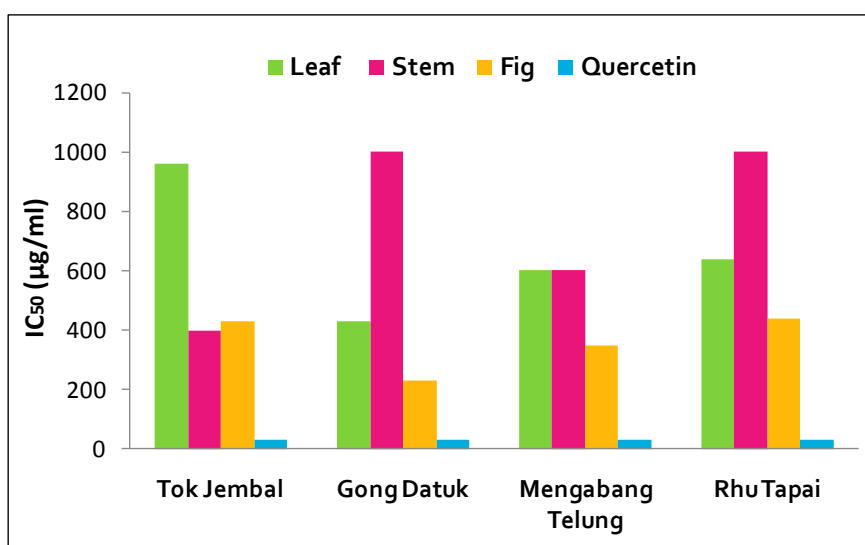


Figure 5. IC_{50} ($\mu\text{g/ml}$) of DPPH antioxidant activity of three plant parts of FDT from four localities around Terengganu areas. Each value represents as the means \pm SE (n=3). There were significant differences ($*p < 0.05$) among three plant parts FDT.

blood glucose, which increase after food intake (post prandial) and therefore can be an important mechanism for the management of carbohydrates metabolic disorders especially in type 2 diabetes [24]. Several alpha-glucosidase inhibitors such as acarbose, miglitol and voglibose isolated from bacterial cultures or their derivatives have been used to reduce glucose after taking meal [25]. However, the use of these drugs as inhibitors is associated with side effects including liver disorder, abdominal distention, flatulence,

meteorism and diarrhea. In such circumstance, medicinal plants were suggested as alternative medicine for prevention and treatment of diabetes because of their negligible side effects [26]. Leaves, stems and figs part of FDT from four localities have shown to have good activity upon alpha glucosidase inhibition assay (Figure 6). Fig extracts from all localities show the highest inhibition of alpha glucosidase enzyme with the lowest IC_{50} value, followed by leaf extracts (Figure 7). This finding shows

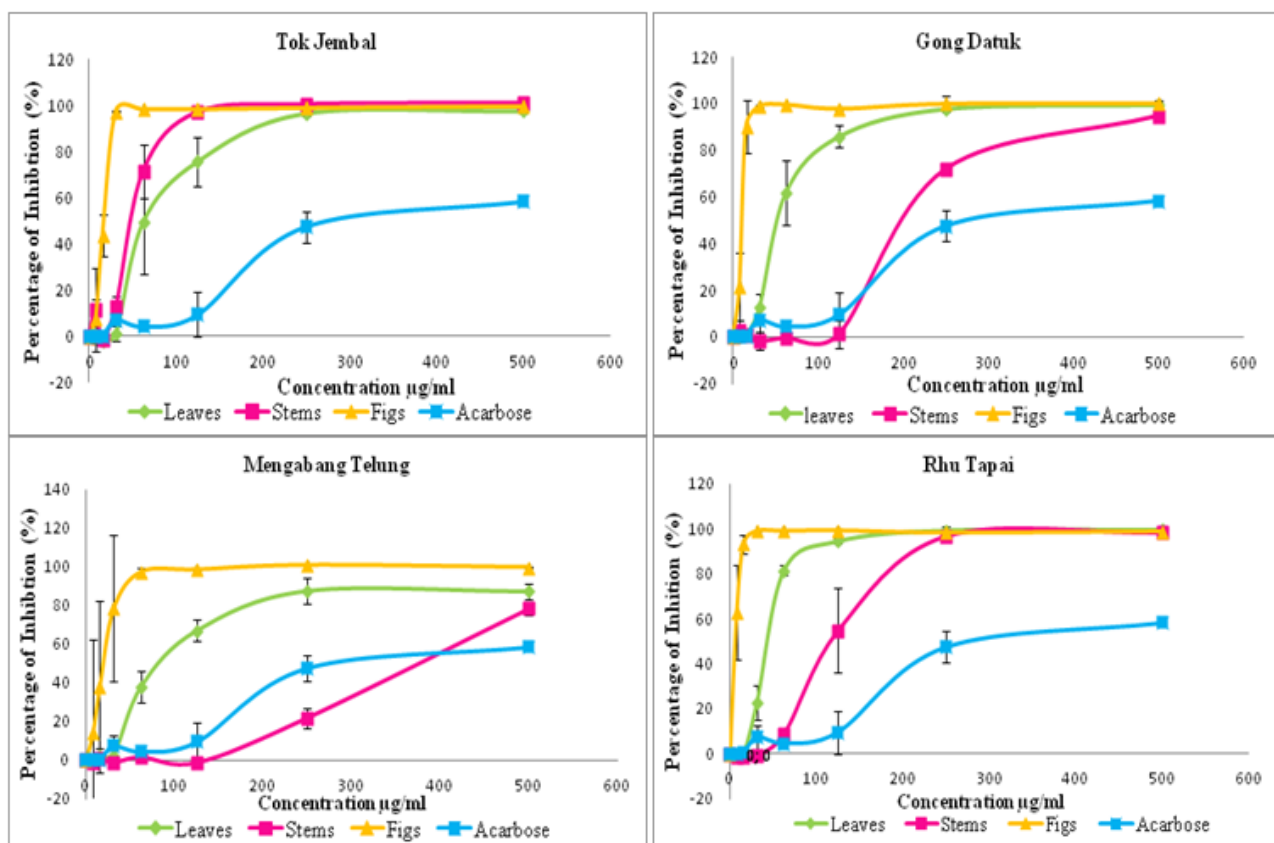


Figure 6: Alpha-glucosidase inhibition assay for plant parts of FDT from four localities around Terengganu areas.

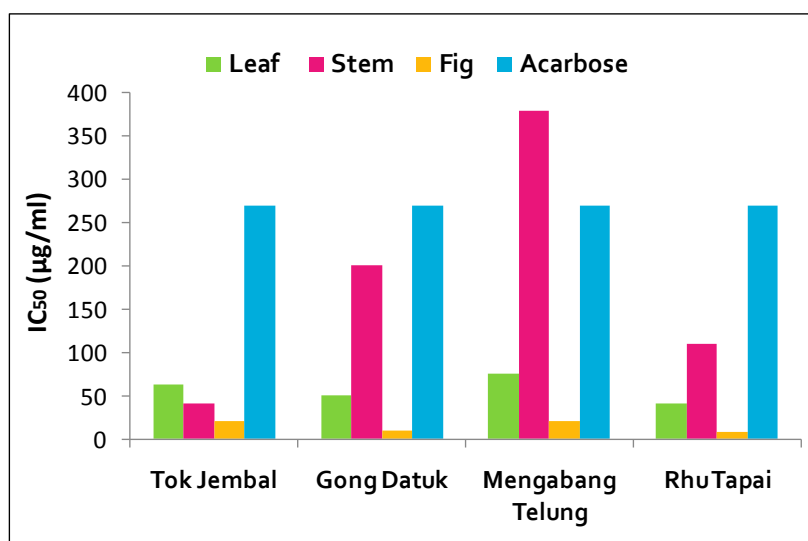


Figure 7: IC₅₀ (µg/ml) of alpha-glucosidase inhibition assay of three plant parts of FDT from four localities in Terengganu. Each value represents as the means ±SE (n=3). The value is significant differences (p< 0.05) between three plant parts of FDT from four localities around using One-way ANOVA (Dunnett's T3) post hoc analysis.

that figs are better part to be consumed at least in the case of antidiabetic activity through alpha glucosidase inhibition.

CONCLUSION

The use of thin layer chromatography in this study provides simple, flexible, fast and inexpensive techniques for separation of the chemical constituents in the samples

extract. The comparison between plant parts of FDT from different markers was carried out based on the separation of different markers, isovitexin and vitexin and it shows variability in term of the intensity of the spots. TLC chromatogram shows that fig have richer chemical components compared to leaf and stem. TLC also revealed that fig extract possesses specific constituents, which could

further be isolated and identified as specific marker for fig of *Ficus deltoidea*.

Chemical fingerprinting by FT-IR analysis coupled with chemometric analysis show the significant discrimination between the plant parts from different localities as shown by clustering group in PCA scores plot. Figs extracts from all localities clustered together indicating the occurrence of specific constituents in its extracts. Biological activity of FDT also support the notion that fig is more biologically active compared to leaf and stem. Taking together, finding from this work revealed that the consumption of fig is a better choice compared to other parts of *Ficus deltoidea* var. *trengganuensis*, especially as antioxidant and anti diabetic therapeutic.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Amirrudin Ahmad and Mr. Syed Ahmad Rizal for collecting the samples. This study was supported by Research Acculturation Collaborative Effort (RACE) Grant Scheme (no. RACE/F1/SG2/UniSZA/1) and Research Acculturation Grant Scheme (RAGS) by Ministry of Education, Malaysia.

REFERENCES

- Mustaffa F, Indurkar J, Ali NIM, Hanapi A, Shah M, Ismail S, Mansor SM. A review of Malaysian plants with potential antidiabetic activity. *Journal of Pharmacy Research* 2011; 4(11):4217-4224.
- Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin, S, Mohamad AS et al. Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. *Fitoterapia* 2008; 79:557-561.
- Hakiman M and Maziah M. Non enzymatic and enzymatic antioxidant activities in aqueous extract of different *Ficus deltoidea* accessions. *Journal of Medicinal Plants Research* 2009; 3(3):120-131.
- Norra I. Free radical scavenging activity and phenolic content of *Ficus deltoidea* accessions MFD4 and MFD6 leaves. *Journal Tropical Agricultural and Food Science* 2011; 39(1): 1-8
- Adam Z, Hamid M, Ismail A, Ismail S. Effect of *Ficus deltoidea* extracts on hepatic basal and insulin-stimulated glucose uptake. *Journal of Biological Sciences* 2009; 9(8): 796-803.
- Adam Z, Khamis S, Ismail A, Hamid M. Inhibitory properties of *Ficus deltoidea* on α -glucosidase activity. *Research Journal of Medicinal Plant* 2010; 4(2):61-75.
- Adam Z, Khamis S, Ismail A, Hamid M. *Ficus deltoidea*: A potential alternative medicine for diabetes mellitus. *Evidence-Based Complementary and Alternative Medicine* 2012; Article ID: 632763:12
- Oh M-J, Hamid AM, Ngadiran S, Seo Y-K, Sarmidi MR, Park CS. *Ficus deltoidea* (Mas cotek) extract exerted anti-melanogenic activity by preventing tyrosinase activity in vitro and by suppressing tyrosinase gene expression in B16F1 melanoma cells. *Arch. Dermatol. Res* 2011; 303:161-170.
- Hasham R, Choi HK, Sarmidi MR, Park CS. Protective effects of a *Ficus deltoidea* (Mas cotek) extract against UVB-induced photoageing in skin cells. *Biotechnology and Bioprocess Engineering* 2013; 18:185-193.
- Corner, E.J.H. The complex of *Ficus deltoidea*; a recent invasion of the Sunda shelf. *Phil. Trans. R. Soc. Lond* 1969; 256:281-317.
- Mohd KS, Azemin A, Hamil MSR, Abu Bakar AR, Dharmaraj S, Hamdan MR, et al. Application of HPTLC and FTIR profiling coupled with chemometrics for the differentiation of the varieties of *Ficus deltoidea* Jack. *Asian Journal of Pharmaceutical and Clinical Research* 2014; 7(5): 110-116.
- Adam Z, Hamid M, Ismail A, Khamis S. Effect of *Ficus deltoidea* aqueous extract on blood glucose level in normal and mild diabetic rats. *Jurnal Sains Kesihatan Malaysia* 2007; 5(2): 9-16.
- Syed Aris SR, Mustafa S, Ahmat N, Mohd Jaafar F and Ahmad R. Phenolic content and antioxidant activity of fruits of *Ficus deltoidea* var. *angustifolia* sp. *The Malaysian Journal of Analytical Sciences* 2009; 13(2): 146-150.
- Khairi HM, Afifah MYN, Somchit MN and Moin S. Anti-inflammatory properties of aqueous fruit extract of *Ficus deltoidea* Jack var. *angustifolia* (Miq.) Corner. *Medicinal and Aromatic Plants Seminar (MAPS 2010) August 3– 4 2010*
- Bunawan H, Mat Amin N, Bunawan SN, Baharum SN and Mohd Noor N. *Ficus deltoidea*: A review on Its Phytochemical Pharmacological Importance. *Evidence-Based Complementary and Alternative Medicine* 2014; 8
- Misbah H, Abdul Aziz A and Aminudin N. Antidiabetic and antioxidant properties of *Ficus deltoidea* fruit extracts and fractions. *BMC Complementary & Alternative Medicine* 2013; 13:118.
- Aminudin N, Sin CY, Chee ES, Nee KI, Renxin L. Blood glucose lowering effect of *Ficus deltoidea* aqueous extract. *Malaysian Journal of Science* 2007; 26 (1):73-78.
- Dzolin S, Aris SSR, Ahmad R. and Mat Zain M. Radical scavenging and neurotoxicity of four varieties of *Ficus deltoidea*. *International Conference on Science and Social Research (CSSR)*. December 5-7 2010, Kuala Lumpur, Malaysia
- Mayur B, Sandesh S, Shruti S. and Sung-Yum S. Antioxidant and α -glucosidase inhibitory properties of *Carpesium abrotanoides* L. *Journal of Medicinal Plants Research* 2010; 4(15): 1547-1553.
- Marston A and Hostettmann K. Separation and Quantification of Flavonoids. In: Andersen Ø. M. and Markham K. R., editor. *Flavonoids: chemistry, biochemistry and applications*. London (NY): CRC Press Taylor & Francis Group; 2006. p. 1-36
- Bougatef A, Hajji M, Balti R, Lassoued I, Triki-Ellouz Y. and Nasri M. Antioxidant and free radical-scavenging activities of smooth hound (*Mustelus mustelus*) muscle protein hydrolysates obtained by

- gastrointestinal proteases. Food Chemistry 2009; 114: 1198-1205.
22. Shimada K, Fujikawa K, Yahara K and Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agriculture and Food Chemistry 1992; 40: 945-948.
 23. Farsi E, Shafaei A, Yee Hor S, Khadeer Ahamed BM, Yam MF, Attitalla IH, Asmawi, MZ, and Ismail Z. Correlation between enzymes inhibitory effects and antioxidant activities of standardized fractions of methanolic extract obtained from *Ficus deltoidea* leaves. African Journal of Biotechnology. 2011;10(67): 15184-15194.
 24. Van de Laar FA, Lucassen PL, Akkermans RP, van de Lisdonk EH, Rutten GE and van Weel C. A-Glucosidase inhibitors for patients with type 2 diabetes. Diabetes Care 2005; 28: 166-175.
 25. Kalra S. Alpha glucosidase inhibitors. J. Pak. Med. Assoc. 2014;64(4): 474-476.
 26. Cheng AYY and Fantus GI. Oral antihyperglycemic therapy for type 2 diabetes mellitus. Canadian Medical Association 2005; 172(2):213-226.