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Anti-inflammatory Activity of Standardised Extracts of Leaves of Three Varieties of *Ficus deltoidea*

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ABSTRACT
Present study aimed to evaluate standardised extracts of different verities of *Ficus deltoidea*, a traditional medicinal plant, for anti-inflammatory activity using three *in vitro* assays, lipoxygenase, hyaluronidase and TPA-induced oedema. Methanol and aqueous extracts were standardised by high performance liquid chromatography (HPLC) using two pharmacologically active markers, vitexin and isovitexin, which were isolated from methanol extract of the plant having code FDT1M. The method was validated and then applied to standardise extracts of the plant. In the extracts, the concentration of vitexin and isovitexin varied in the range 2.45 ± 0.00 - 19 ± 0.12 mg/g and 1.58 ± 0.02 - 41.49 ± 0.47 mg/g, respectively. Different extracts of three varieties of the plant displayed different anti-inflammatory activities ($p < 0.05$). The activity of the extracts was found comparable to apigenin, nordihydroguaiaretic acid, indomethacin, which were used as control ($p < 0.05$). The results of this study indicate that extracts of leaves of *Ficus deltoidea* possess anti-inflammatory properties.

Keywords: *Ficus deltoidea*; Standardization; anti-inflammatory, lipoxygenase; hyaluronidase.

INTRODUCTION

Standardization herbal products, consistency in claimed efficacy of a product and its batch-to-batch reproducibility, is a difficult task and is a main hindrance in their wider acceptance. In order to bring these remedies into the mainstream pharmaceutical market, solid scientific evidence is needed to support the efficacy claims of these products. The use of markers, the chemical compounds characteristic of a plant, signifies the total active constituents of an extract or correlates to pharmacological activity are used to standardise herbal products. [1-2] Ficus species contain compounds such as flavonoids [3-8], α-tocopherol and its derivatives [9], steroids and triterpenoids [10-15] and alkaloids. [11, 16-18] Polyphenolic compounds have shown antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic and immune-stimulating properties. [19] Therefore, in this study we selected two C-glycosylflavones, vitexin and insovitexin, as pharmacologically active analytical markers to standardise the extracts of three verities of *Ficus deltoidea.* Markers are in small quantities as compared to the dose used hence, it may be beneficial to know about primary metabolites of the extract. Therefore, the extracts were also analysed for primary metabolites such as total proteins and polysaccharides, which also contribute in activity. The standardised extracts were then evaluated for anti-inflammatory properties.

Ficus deltoidea Jack (*Moraceae*) is found in tropical and sub-tropical countries and has several varieties. [20] The leaves of the plant are used traditionally for treating diabetes, high blood pressure, heart problems, gout, diarrhea, pneumonia and skin diseases. [21] The plant has also exhibited antioxidant, hypoglycemic and antinociceptive properties. [22-23] Zunoliza *et al.*, (2009) [23] reported the presence of high content of total polyphenols, flavonoids and tannins in extracts of leaves of three verities of the plant which have shown good antioxidant activity. Polyphenols have shown antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic and immune-stimulating properties. [19] Therefore, it was hypothesized that extracts of the plant having high content of polyphenols might have anti-inflammatory effects. To test this hypothesis, present study aimed to evaluate aqueous and methanol extracts of leaves of three verities of the plant for anti-inflammatory activities.

Inflammation is a complex process involving several biochemical changes and mediators. [24] Arachidonic acid, a substrate for the biosynthesis of a range of bioactive eicosanoids, is metabolised by the cyclooxygenase (COX) pathway to prostaglandins and thromboxane A2, or by the

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lipoxygenase (LOX) pathway to hydroperoxy-eicosatetraenoic acids and leukotrienes, which are important mediators in a variety of inflammatory events. [25] Several steroidal and non-steroidal anti-inflammatory drugs are based on the inhibition of these pathways and are used to treat inflammatory conditions. Long-term use of these agents is associated with various severe adverse effects. Therefore, natural drugs with little side-effects are required to substitute these drugs. Keeping it in view, present study aimed to evaluate standardised methanol and aqueous extracts of leaves of three verities of *Ficus deltoidea* for anti-inflammatory activity using different *in vitro* models.

MATERIAL AND METHODS

Chemicals

Chemical used in this study included: soybean lipoxygenase, hyaluronidase, hyaluronic acid, apigenin, 12-O-tetradecanoylphorbol-13 acetate (TPA), indomethacin, vitexin (Sigma-Aldrich Chemical Co.), acetonitrile and methanol HPLC grade (Fisher) and ortho-phosphoric acid (Merck). Isovitexin, previously isolated from the plant, was used.

Plant material

Three varieties of *Ficus deltoidea* leaves were selected in this study included *F. D. var. terengganuensis* (2 samples encoded as FDT1 and FDT2), *F. D. var. angustifolia* (FDA) and *F. D. var. deltoidea* (FDD). Sample of FDT1 was obtained from Malai Herbal Tea, Malaysia. FDT2 and FDA were collected from Terengganu and Selangor, respectively. Sample of FDD was purchased from Nutreeherbs Sdn. Bhd. Malaysia. All the samples were authenticated by Ms Zainon Abu Samah, a botanist, Medicinal Plants Program, Biotechnology Division, Forest Research Institute Malaysia (FRIM), where voucher specimens having numbers FRI 48988 for FDT2 and FRI 54761 for FDA were deposited.

Preparation of extracts

Dried and ground leaves were extracted with methanol using Soxhlet extractor for 17 h to get methanol (M) extracts, which were then dried in vacuum at 40°C. To prepare aqueous (W) extracts, dried ground and pulverized leaves were macerated with water at 50°C for 3 h, the procedure was repeated twice and the extracts were filtered and dried in freeze dryer. The extracts were encoded as FDT1M, FDT1W, FDT2M, FDT2W, FDAM, FDAW, FDDM and FDDW, where M and W referred to methanol and water, respectively.

Instrumentation and Chromatographic conditions

The samples were analysed using HPLC system consisting of HP-1100 Agilent Technologies equipped with a quaternary pump, on line degasser, auto-sampler, column heater and UV detector. Chromatographic separations of the C-glycosylflavones were performed on a Lichrospher RP-18 column (125 mm x 4 mm, 4 µm) which was protected by RP-18 guard column (5 µm, 4.0 mm, 3.0 mm i.d.). The temperature of the column was maintained at 35°C. A gradient elution consisting of solvent A (0.1% ortho-phosphoric acid) and solvent B (acetonitrile) was used at flow rate of 1 ml/min as: 0 – 6 min (15 - 25% of A); 6 – 11 min (25% A); 11 – 14.50 min (25 – 15% A) and 14.50 – 23 min (15% A). The injection volume was 10 µl and the effluent was monitored at 335 nm.

Preparation of standard solutions

Stock solutions of vitexin and isovitexin were prepared in methanol to get concentration of 500 and 1000 µg/ml, respectively. Mix standard stock solution was prepared from the stock solutions of vitexin and isovitexin to get the concentration of 250 and 500 µg/ml, respectively. Working mix standard solutions were prepared by further diluting the mix standard stock solution with methanol to a concentration of 0.01-250 µg/ml of vitexin and 0.01-500 µg/ml of isovitexin.

Validation of HPLC method

The linearity of calibration curves was evaluated by linear regression analysis and correlation coefficient (R^2). The limit of detection (LOD) and limit of quantification (LOQ) were evaluated by measuring the magnitude of analytical background by injecting the blank. In this study, LOD were determined by injection a series of solutions until the height of the peak signal to baseline noise level ratio (S/N) was 3: 1, whilst LOQ values were taken at S/N 10: 1.

The accuracy and precision of the method were determined through within-day and between-day analysis of mix standard solutions. A separate standard curve was constructed on each day of analysis. The within-day accuracy and precision were determined for each compound on three concentrations with five replicate on a single day, while the between-day accuracy and precision was carried out over five consecutive days. The accuracy was expressed as percent of true value and respective precision was expressed as relative standard deviation (% RSD).

The accuracy of extraction was evaluated through recovery studies by spiking the leave powder with three different concentration of standard solution. The accuracy was calculated with the value of detected versus added amounts.

Preparation of samples and their analysis

Methanol extracts were reconstituted with methanol to a concentration of 10 mg/ml, whereas aqueous extracts were dissolved in 50 % methanol to produce solutions of concentration 10 mg/ml. All the samples were filtered through a 0.45 µm filter (Whatman) and kept in HPLC vials.

All the samples were analysed in triplicate by HPLC using analysis conditions mentioned above and markers were quantified from the standard curves.

Determination of Total Protein

Protein concentration in the extracts was measured according to the method of Lowry *et al.*, (1951) [26] using Bovine Serum Albumin (BSA) as a standard. Stock solution of BSA was prepared in distilled water (500 ppm). A series of dilutions (50, 100, 150 and 200 ppm) were prepared at final volume of 1 ml. Buiret reagent (3 ml) prepared by mixing 50 ml f 2 % sodium carbonate in 0.1 N sodium hydroxide and 1 ml of 0.5% copper sulphate in 1 % potassium tartrate was added to standard solution (1 ml) and sample solution 1 ml having concentration 500 ppm. The mixture was allowed to stand for 10 min at room temperature. Then 200 µl Folin-Ciolcateau reagent was added and reaction mixture was allowed to incubate for 10 min at room temperature, and absorbance was measured at 600 nm. Protein content was determined from the calibration curves of BSA.

Determination of Total Polysaccharide

Total polysaccharide content was determined according to the method of Adams and Emerson (1960) using glucose as a standard. [27] Briefly described as the extract was washed with 80% of hot ethanol by centrifugation at 3000 rpm for 10 min and the procedure was repeated until the washing did not give color with anthrone reagent. The residue was then dried over water bath and extracted with 5 ml distilled water and 5

ml of 25 % HCl for 20 min at 0°C. The tube was then centrifuged at 3000 rpm for 10 min and the supernatant was transferred to 100 ml volumetric flask. The extraction was repeated in the same way and the supernatant was poured into the same volumetric flask and the volume was made 100 ml with distilled water. For analysis, 100 µl of the supernatant was taken into a test tube and made up the volume 1 ml with distilled water. Glucose stock solution (1000 ppm) was prepared in distilled water and a series of working standards solutions of concentration 20, 40, 60, 80, and 100 ppm were prepared to final volume of 1 ml. Anthrone reagent (4 ml) was added to standards and extract solutions and the reaction mixture was heated for 8 min in a boiling water bath. Test tubes were then cooled rapidly and the absorbance was measured at 630 nm against distilled water as a blank. Calibration curve of glucose was used to determine the total amount of polysaccharide in the samples.

Anti-inflammatory activity by Lipoxygenase inhibition assay

Lipoxygenase inhibiting activity was measured using spectrophotometric method. [28] Test samples and reference standards were dissolved in methanol. The reaction mixture was prepared in 96-well microplate by adding sodium phosphate buffer 160 µl, test sample solution 10 µl and soybean lipoxygenase solution 20 µl. Then reaction was initiated by adding sodium linoleic acid solution 10 µl, which acted as substrate. The enzymatic conversion of linoleic acid was measured at 234 nm over a period of 6 min. All the reactions were performed in triplicate.

Anti-inflammatory activity by hyaluronidase inhibition assay

The assay was performed according to Sigma protocol with slight modifications. [29] The assay medium consisting of 1.00-1.67 U hyaluronidase in 100 µl of 20 mM sodium phosphate buffer was incubated with 5 µl of the test compound (in DMSO) for 10 min at 37 ^{o}C. Then the assay was commenced by adding 100 µl hyaluronic acid and incubated for further 45 min at 37^{o}C. The undigested hyaluronic acid was precipitated with 1 ml acid albumin solution. After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The absorbance in the absence of enzyme was used as reference value for maximum inhibition. The inhibitory activity of the sample(s) was calculated as the percentage ratio of the absorbance in the presence of test compound versus absorbance in the absence of enzyme. The performance of the assay was verified using apigenin as a reference under exactly the same experimental conditions.

Anti-inflammatory activity by 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced ear oedema

TPA induced mouse ear oedema inhibitory assay was used in the determination of anti-inflammatory activity. The experiment was carried out according to a method described by De Young *et al.*, (1989) and Carlson *et al.*, (1985). [30-31] The animals were grouped randomly each having 5 animals. TPA in acetone (20 µl of 0.05 µg/µl) was applied topically on right ear of each mouse. The test sample dissolved in acetone was applied topically to the inner surface of the right ear (2 mg/ear) about 30 min before each TPA application. The sample vehicle in acetone was applied on the other ear, which served as control. Indomethacin was used as standards. The resulting oedema was measured 6 h after TPA treatment. The results were expressed as percentage inhibition till complete suppression of erythema. Each value used was the mean of individual determinations from 5 mice. Percentage inhibition (IE %) of the test sample was calculated as the ratio of the weight increase of the ear sections. The study protocol was approved by the Animal Ethical Committee of the School of Pharmaceutical Science, Universiti Sains Malaysia.

Statistical analysis

For standardization all the samples were analysed in triplicate and results were averaged. Anti-inflammatory activity study was determined triplicate, except for TPA assay where results are average of 5 replicas. Results of anti-inflammatory activity of different extracts were analysed by one way ANOVA and $P < 0.05$ was regarded as significant.

RESULTS AND DISCUSSION

Vitexin (8-C-glucosyl apigenin) and isovitexin (6-C-glucosyl apigenin) have several pharmacological activities such as anti-hypertensive, anti-inflammatory, antispasmodic, antimicrobial and antioxidant. [32-34] Hence, these compounds have been selected as analytical markers to standardise aqueous and methanol extracts of leaves of three verities of *Ficus deltoidea*. The chemical structures of the markers are presented in Fig. 1.

The chromatographic conditions were optimized to obtain chromatograms with better resolution of adjacent peaks in a shorter time. Various mixtures of water and methanol were used as mobile phase but the separation was not satisfactory. However, when the methanol was replaced by acetonitrile, the separation and resolution was improved. Addition of acid in mobile phase was found to enhance the resolution and eliminate the peak tailing of the target markers. As a result, a gradient elution combining acetonitrile and water containing 0.1 % ortho-phosphoric acid was chosen to get the desired separation. The detection was better at 335 nm as compared to 270 nm. This optimization produced well-resolved peaks of vitexin and isovitexin in a total run of 23 min. The chromatograms the mixed standard solution and the extracts are given in Fig. 2.

The method was found linear over the whole range of markers investigated; vitexin showed good linearity with a regression equation ($Y = 19.809X - 26.599$ with $R^2 = 0.9997$), while isovitexin showed a linearity with regression equation ($Y = 21.253X - 32.96$ with $R^2 = 0.9999$). For both the markers, LOD and LOQ were found to be 1 and 3 µg/ml, respectively. For repeatability and reproducibility of the method, results indicated that the within-day and between-day RSD values of both the compounds were less than 5.58 %, which indicated good repeatability and reproducibility. The extraction recovery was found to be in the range of 87.29 – 116.58 % with R.S.D less than 5.02 %. These results indicate that the method is repeatable and reproducible because results were not compromised in within and between day analysis.

This validated method was applied to standardise methanol and water extracts of leaves of three varieties of *Ficus deltoidea*. Peaks were identified by comparing the retention times. Methanol and aqueous extracts from *Ficus deltoidea* var. *terengganuensis* showed several non-identified peaks at retention time different from the standards. Both the samples of *Ficus deltoidea* var. *terengganuensis* showed similar HPLC chromatograms but vary in the concentration of vitexin and isovitexin. The HPLC profiles of extracts of

Table 1: Content of markers (vitexin and isovitexin) and total polysaccharide and total protein in the aqueous and methanol extracts of *Ficus deltoidea,* (n = 3)

Extracts	Vitexin (mg/g)	Isovitexin (mg/g)	Polysaccharides (%)	Protein (%)
FDT1M	4.95 ± 0.12	7.84 ± 0.18	0.9548 ± 0.06	14.9164 ± 0.88
FDT1W	2.45 ± 0.00	3.61 ± 0.01	2.1402 ± 0.08	47.9373 ± 1.22
FDT2M	5.69 ± 0.09	12.78 ± 0.20	0.9657 ± 0.04	35.3691 ± 1.35
FDT2W	19.00 ± 0.12	20.49 ± 0.09	2.7570 ± 0.27	66.5318 ± 2.74
FDAM	12.18 ± 0.15	41.49 ± 0.47	0.9984 ± 0.09	18.9127 ± 0.77
FDAM	6.60 ± 0.09	26.94 ± 0.26	5.1059 ± 0.17	13.6691 ± 0.20
FDDM	7.30 ± 0.15	1.58 ± 0.02	0.0006 ± 0.00	6.3891 ± 0.26
FDDW	5.48 ± 0.01	nd	0.007 ± 0.00	34.6926 ± 0.79

(FDT1M), (FDT1M), (FDT2M) and (FDT2W) are extracts of *Ficus deltoidea* var. *terengganuensis*; (FDAM) and (FDAW) are extracts of *Ficus deltoidea* var. *angustifolia*; (FDDM) and (FDDW) are extracts of *Ficus deltoidea* var. *Deltoidea,* nd (not detected)

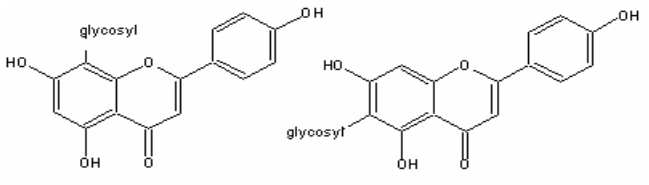

Fig. 1: Chemical structure of two C-glycosylflavones, vitexin (1) and isovitexin (2)

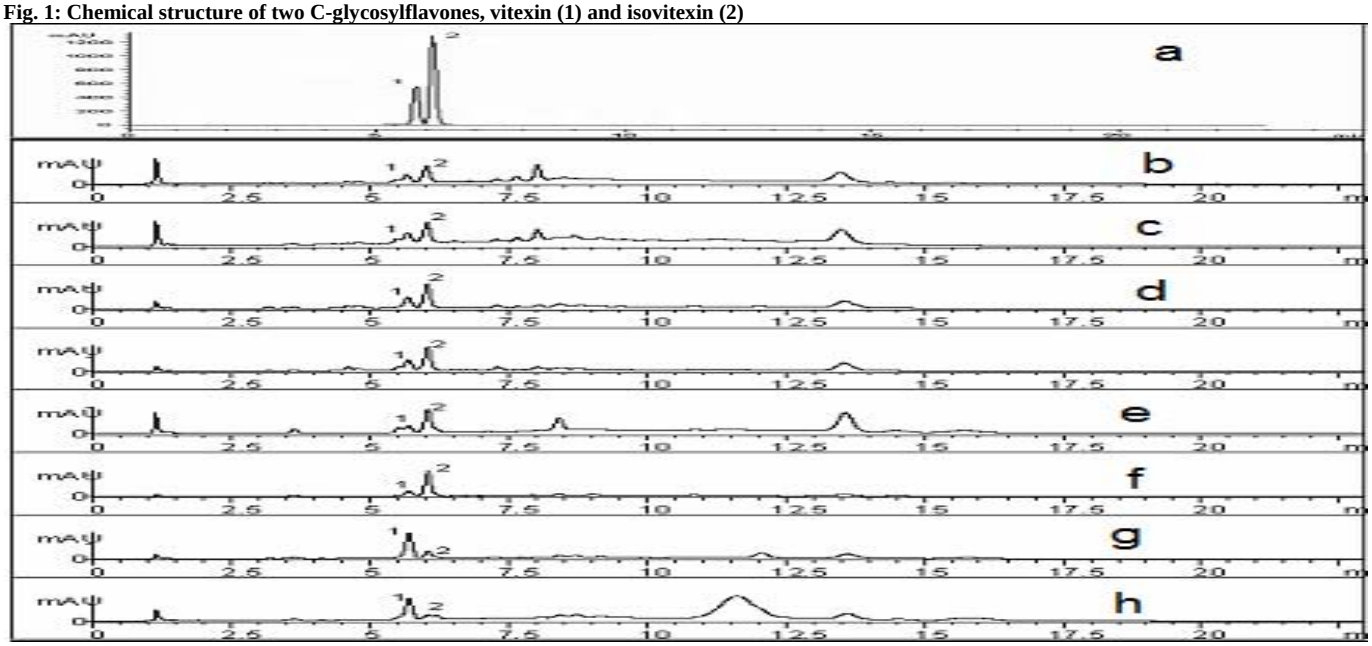

Fig. 2: Chromatograms of mix standard solution, methanol extracts (M) and aqueous extracts (W) detected at 335 nm, a (mix standard solution); a (FDT1M), b (FDT1M), c (FDT2M) and d (FDT2W) are extracts of *Ficus deltoidea* var. *terengganuensis*; e (FDAM) and f (FDAW) are extracts of *Ficus deltoidea* var. *angustifolia*; g (FDDM) and h (FDDW) are extracts of *Ficus deltoidea* var. *deltoidea*; (1) vitexin; (2) isovitexin

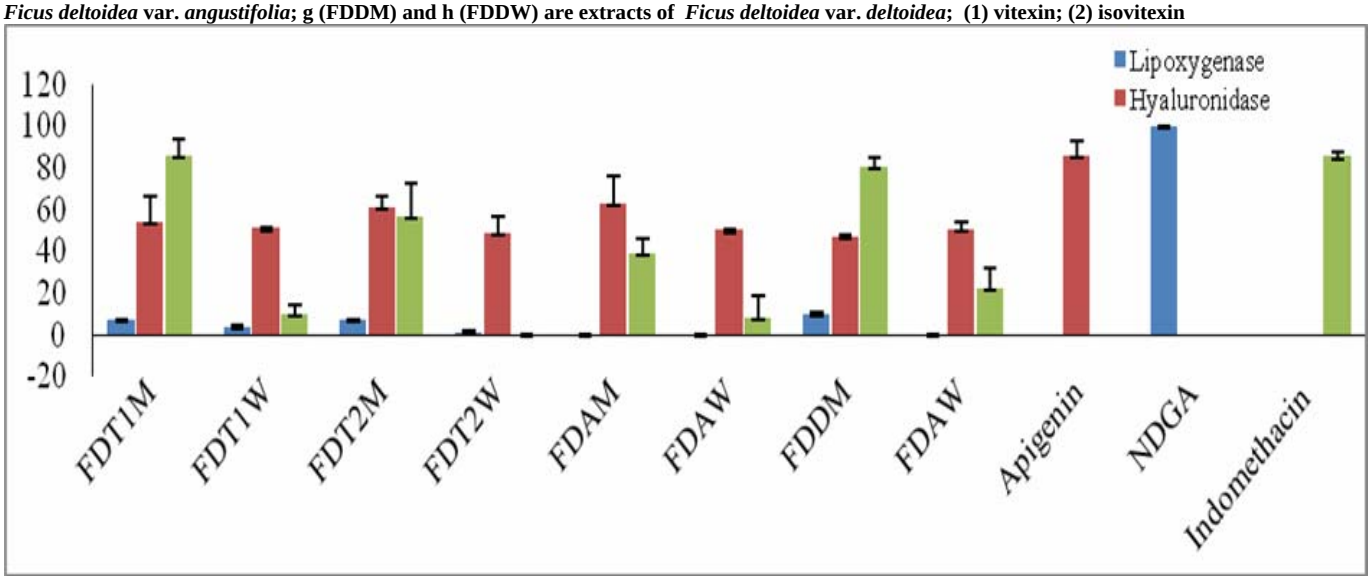

Fig. 3: Anti-inflammatory effects of aqueous and methanol extracts and standards on LOX, , HAase and TPA-induced ear oedema, (FDT1M), (FDT1M), (FDT2M) and (FDT2W) are extracts of *Ficus deltoidea* var. *terengganuensis*; (FDAM) and (FDAW) are extracts of *Ficus deltoidea* var. *angustifolia*; (FDDM) and (FDDW) are extracts of *Ficus deltoidea* var. *Deltoidea;* NDGA (nordihydroguaiaretic acid); Each value represents mean ± SD (n = 3);* *P* < 0.05 (a significant difference as compared to nordihydroguaiaretic acid); *P* < 0.05 (b significant difference as compared to apigenin); *** *P* < 0.05 (c significant difference as compared to indomethacin)**

Ficus deltoidea var. *angustifolia* and *Ficus deltoidea* var. *deltoidea* showed only a few peaks as compared to chromatographic profiles of *Ficus deltoidea* var. *terengganuensis*. From the results, isovitexin was found to be main chemical component in *Ficus deltoidea* var. *angustifolia*, while vitexin was found as the main chemical component in *Ficus deltoidea* var. *deltoidea*. The content of both the markers in different extracts of three verities are presented in Table 1. The concentration of vitexin and isovitexin in these extracts varies in the range 2.45 ± 0.00 to 19 ± 0.12 mg/g extracts and 1.58 ± 0.02 to 41.49 ± 0.47 mg/g extracts, respectively.

All aqueous and methanol extracts were evaluated for the bioactive compounds, total polysaccharide and protein (Table 1). These results indicated that percentage of protein in water extracts ranged from 13.67 - 66.53 %, while the percentage of protein in methanol extracts ranged from 6.39 - 35.37 %. The percentage polysaccharide in all extracts was less than 5.11 %.

The results of lipoxygenase inhibitory properties of the extracts using 15-soybean lipoxygenase are presented in Fig. 3. It is obvious from the results that all the extracts showed low LOX inhibition activity as compared to the standard, nordihydroguaiaretic acid (100 % inhibition). Among these extracts, aqueous extracts of FDD and methanol extract of FDA exhibited zero LOX inhibition activity, whereas FDDM, FDT1M and FDT2M exhibited 10.35 ± 0.04 %, 7.55 ± 0.04 % and 7.60 ± 0.01 % inhibition, respectively. These results showed that the extracts did not display anti-inflammatory activity via LOX mechanism.

According to Guo *et al.* (2002) [35] antioxidants display anti-inflammatory effects because the level of free radicals is increased in inflammation. Phenolic compounds have the potential to block the process of arachidonic acid metabolism by inhibiting lipoxygenase activity. However, according to Rackova *et al.* (2007) [36] scavenging effect of free radical may not be a critical factor behind the inhibition of LOX pathway rather inhibition is suggested to be due to the specific interaction of constituents with the enzyme. An inhibition of the LOX can be achieved via chelation of its non-heme bound iron [37] or by reduction of its ferric form. [38-40] It explains the disability of the extracts to inhibit LOX, though these extracts have shown antiradical properties. [23] An interaction with iron atom at the enzyme catalytic centre may be involved in the LOX inhibition mechanism.

Results of hyaluronidase (HAase) inhibition of methanol and aqueous extracts presented in Fig. 3 indicated that the extracts exhibited moderate inhibitory activity against HAase, which was comparable to that of the standard, apigenin (85.60 ± 7.15 %). The data obtained, showed that methanol extracts of sample FDT1, FDT2 and FDA have slightly higher HAase inhibition activity than the aqueous extracts. In contrary, aqueous extracts of FDD exhibited slightly higher percentage of HAase inhibition activity as compared to methanol extracts. The HAase inhibition activity of methanol and aqueous extracts varied from 47.05 - 62.95 % and 48.97 - 51.0 %, respectively. Except FDDM extracts, activity of all the extracts was significantly different as compared to standards ($P < 0.05$). The results indicated that these extracts could be considered moderate inhibitor of HAase.

The presence of protein in the assay mixture effect the release of HA by HAase and the activity is decreased with the increase of protein concentration. [41] The mechanism by which low concentration of proteins enhances the enzyme activity is unclear but it may be hypothesized that the polyanionic HA molecule can bind to the small amounts of proteins that facilitate the opening of the HA random coil, thus facilitating hyaluronidase accessibility to the HA. Most recent study by Descherevel *et al.* (2008) [42] on the effects of added non-catalytic protein, bovine serum albumin (BSA), on the HAase activity showed that the addition of BSA induces competition with HAase to form non-specific complex with HA, thus consequently releases free HAase which is catalytically active leading to an increase in hydrolysis of HA. The study indicates that the stability of the non-specific complex of HA-BSA was higher than that of HA-HAase suggesting that as long as the added BSA releases HAase by forming non-specific complex with HA, it will induce an increase in the hydrolysis of HA that is catalyzed by HAase. Under these conditions, BSA acts as activator of HAase. However, further addition of BSA increases the formation of HA-BSA complexes, which results in lowering the initial rate. In this case, the formation of HA-HAase complex is hindered, and BSA acts an inhibitor of HAase.

Therefore, the content of protein in the extracts should be considered in the hyaluronidase activity. In this study, the total of protein content has been estimated and presented in Table 1. As mentioned earlier, the methanol extracts have slightly higher percentage of HAase inhibition as compared to aqueous extracts. Conversely, the protein content of the aqueous extracts is slightly higher than the methanol extracts. This may explain the results of moderate percentage of HAase inhibition, thus suggesting the involvement or the interactions of protein with the other chemical constituents of the extracts in this activity.

Many polyanions, such as glycosaminoglycans (heparin, heparin, sulphate, dermatan sulphate), HA derivatives (O-sulpfonated HA) and synthetic polyanions (polystyrene-4-sulfonate) are known to inhibit HAase. [42] Formation of HA-polycation complexes (polycationic polysaccharides) indirectly inactivates HAase by hindering HAase accessibility to HA. [42] Total polysaccharide content of extracts is presented in Table 1. The total polysaccharide content in each extracts may affect the HAase activity. However, the percentage of total polysaccharides in these extracts is quite low as compared to the total protein in respective extracts, hence may only contribute to a small extenct in HAase activity.

Results of TPA-induced mice ear oedema shown in Fig. 3 indicated that methanol extracts exerted noteworthy activity as compared to the aqueous extracts. This may indicate the presence of active substances endowed with anti-inflammatory activity. As observed from Fig. 3, FDT1M, FDT2M, FDDM and FDAM exhibited moderate to strong reduction of oedema ($P < 0.05$) with percentage inhibition of 85.46 ± 8 %, 56.56 ± 16 %, 80.46 ± 4 % and 38.74 ± 7 %, respectively, and the results were comparable to indomethacin, a reference compound that had shown 85.4 ± 2% inhibition. However, aqueous extracts exhibited low inhibition activity with 8.38 ± 10 %, 10.28 ± 4 % and 22.53 ± 9 % for FDDW, FDT1W, FDAW, respectively, whereas FDT2W was found completely inactive in this model. This might be due to the high total polyphenols contained in the methanol extracts as compared to the aqueous extracts. Among aqueous and methanol standardised extracts of leaves

of three varieties of *Ficus deltoidea*, methanol extracts exhibited potent anti-inflammatory activity in three models; lipoxygenase (LOX), hyaluronidase (HAase) and TPA-induced ear oedema. Among these models, the anti-inflammatory activities of methanol extracts were more in TPA model.

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