



## APPLICATION OF ULTRASOUND ON THE EXTRACTION OF VITEXIN FROM FICUS DELTOIDEA LEAVES

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### ABSTRACT

*Ficus deltoidea* or Mas cotek is one of the most popular plant herbs that have been widely used traditionally as postpartum treatment and health tonic. Preliminary or existing extraction method has been used to extract the plant herbs that have low productivity range. This method however can still produce the desired products, but the work can be laborious and not efficient in large scale processing. Besides, the sample preparation has no evidence in advances because obtaining such extraction products at sufficient concentrations usually involves extraction with large amounts of organic solvents and toxic, followed by evaporation. Production of Vitexin from *Ficus deltoidea* extraction under various sonication regiments is reported. Batch extractions were carried at low intensity sonication ( $8.66 \text{ W cm}^{-2}$  sonication intensity at the sonotrode tip) using 10%, 20% and 40% duty cycles. (A duty cycle of 10%, for example, was equivalent to sonication for 1 s followed by a rest period (no sonication) of 10 s.) The extracts were compared with those obtained by conventional boiling extraction, in terms of bioactive constituents yield and chemical composition. In the aqueous (conventional) extracts, the actual percentage weight of Vitexin varied in the range  $0.122 \pm 0.013 \sim 0.386 \pm 0.018$  (%w/w) compared to sonicated extracts with 2-fold increases at a range of  $0.127 \pm 0.001 \sim 0.738 \pm 0.050$  (%w/w) after 8 hours of extraction with sample-to-water ratio of 10:1 (g/ml) at 50, 70 and  $100^{\circ}\text{C}$ . Both ultrasonic-assisted and aqueous extracts were characterized and standardized by HPLC-diode array detector using a pharmacologically active marker, Vitexin. The experimental values under optimal conditions were in good consistent with the predicted values, which suggested that ultrasonic-assisted extraction (UAE) is more efficient process as compared to conventional boiling extraction (AE). It recommends that ultrasound extraction of *F. deltoidea* leaves is substantially feasible to replace the traditional time-consuming and low efficiency preparation procedure in the future modernized and commercialized manufacture of this highly valuable herbal medicine.

**Keywords:** *Ficus deltoidea*, Mas cotek, ultrasound, Vitexin, HPLC.

### INTRODUCTION

In traditional medicine, *Ficus deltoidea* has had a lengthy history of use among Malays to diminish and heal affliction such as rheumatism, sores and wounds and as an after-birth tonic and antidiabetic drug. The range of published extraction of active constituents for this plant include the determination of phenolic content, antioxidant activity (Ruzaina *et al.* 2009; Wahid *et al.* 2010), isolation of moretenol (Lip *et al.* 2009) and identification of Vitexin and Isovitexin (Choo *et al.* 2012). Instead of its antioxidant activity, this *Ficus* species also shows some others medicinal properties such as being anti-bacterial, anti-diarrhoea, anti-diabetic, anti-inflammatory and anti-ulcer (Wahid *et al.* 2010). Preliminary extraction method of this plant using the aqueous extract has been reported by Sulaiman *et al.* (2008) and well-known as decoction.

In Malaysia, the water extract of this plant herb has been widely used for women health. According to Abdul Hamid *et al.* (2011), this water extract has been used for a long time by women who is preparing or after giving birth and as a postpartum treatment to help in contracting the muscles of the uterus and in the healing of the uterus and vaginal canal. Furthermore, the powdered root and leaves of *F. deltoidea* have been used to treat wounds, rheumatism, sores and other ailments for centuries. The decoction of boiled leaves of this plant also is traditionally used as an antidiabetic treatment,

menstrual cycle disorders treatment and to treat leucorrhoea. Additionally, *F. deltoidea* fruits are traditionally chewed to relieve toothache, cold and headache, while the entire plant is used as an aphrodisiac tonic in Indonesia (Bunawan *et al.* 2014).

Misbah *et al.* (2013) claimed that some of the compounds present in *F. deltoidea* are tannins, proanthocyanidins, triterpenoids and phenols (Omar *et al.* 2011), and also flavonoids of Vitexin and Isovitexin (Farsi *et al.* 2011; Choo *et al.* 2012). Besides, the tea extracted from this herb showed no signs of toxicity based on sub-acute toxicity study and has high potential in reducing total cholesterol and risk of cardiovascular disease (Hadijah *et al.* 2007). In order to examine the medicinal properties of *F. deltoidea*, several extraction methods were established by using aqueous or water extraction (Wahid *et al.* 2010; Misbah *et al.* 2013; Abdul Hamid *et al.* 2011) and solvent extraction using methanol (Woon *et al.* 2013; Abdullah *et al.* 2009) and also other types of solvents such as hexane and chloroform (Ruzaina *et al.* 2009).

Vitexin (apigenin-8-C-glucoside) is one of the remarkable compounds in flavone C-glycosides that appeared as light yellow powder form. The flavone C-glycoside is one of an important subclass of the flavonoids family because it usually presents in foodstuffs and nutraceuticals. They are also reported to exhibit anti-



inflammatory and anti-nociceptive properties. Nowadays, they have received much attention because of their antioxidant and anticancer properties.

Numerous works exist on using the decoction to break or damage the cell wall of cellulosic plant, especially of *F. deltoidea* (Abdul Hamid *et al.* 2011; Wahid *et al.* 2010; Sulaiman *et al.* 2008). However, few studies have attempted to use ultrasound to enhance the productivity extraction of active compounds.

Ultrasound as a form of energy can be used to influence or change the nature of the medium. Some interactions caused by ultrasound vibration and the medium included the thermal effects, mechanical effects and cavitation (Xiao *et al.* 2005). Compared to the conventional extraction methods, ultrasonic technology extraction of alkaloids, flavonoids, polysaccharides, saponins, anthraquinone, coumarin and other biologically active compounds from plants costs less time, promotes high yield and has almost no effect on its physiologically active. Some studies reported that the flavonoids content and yield detected increases with increasing of ultrasound power from 50 – 120W (Zhang *et al.* 2008; Xiao *et al.* 2005; Ming *et al.* 2013). However, at some point, the flavonoid content is decreased with ultrasound power more than 120W due to the enzyme inactivation (Ming *et al.* 2013). In terms of reaction time, the first 30 minutes of sonication is more efficient and gives optimize yields (Zhang *et al.* 2008). While Ming *et al.* (2013) stated that the optimum yield can be achieved at 59 minutes of sonication in continual or intermittent sonication.

So far, there seems no report about ultrasound-assisted extraction from *F. deltoidea* leaves. This study was aimed to determine the effects of some factors on the yield of Vitexin with or without the ultrasound treatment. The results from UAE were compared with that obtained from the conventional extraction (control).

## MATERIALS AND METHODS

### Preparation of Plant Material

Dried leaves of *Ficus deltoidea* or Mas Cotek (var. *deltoidea*, 4 kg) were bought from a plantation in Muadzam Shah, Pahang, Malaysia. According to Dzolin *et al.* (2010), the aqueous extract of *F. deltoidea* leaves from this variety contained a significant higher amount of total phenolics compared to other varieties of leaves extract. While Hakimian and Maziah (2009) claim that the female leaf extracts of *F. deltoidea* scavenge more free radicals compared to the male leaf extracts. The dried leaves are sent to the milling factory to be further dried, cleaned and ground into small particles. The leaves powder was kept in a freezer at - 4°C until experiments were on. The uniformity of particle size was determined by sieving the leaves powder through an 850 µm (ASTM No. 20, 20 Mesh) standard sample sieve and a sieve shaker. This particle size was chosen based on a study conducted by Kamal *et al.* (2008) that claims the total antioxidant capacity (TAC) of tea infusion was the highest when using the tea powder at particle size of 850

µm – 500 µm and 1 mm – 500 µm.

### Conventional Extraction

Sample was mixed with distilled water in sample-to-water ratio of 10:1. Volume of infusion was set at 300 ml for each mixture ratio in order to eliminate the volume differences. The mixtures were heated on a hotplate (IKA, Germany) at 50.0, 70.0 and 100.0 °C for 8 hours with uniform stirring. 10 ml of extract was sampled every hour. 500 ml beaker containing the mixture was used and covered with aluminium foil throughout the extraction to minimize the evaporation in order to maintain the sample-to-water ratio (Wahid *et al.* 2010). Extraction sample was left to cool at room temperature and centrifuged (Kubota, Japan) at 5800 rpm for 15 minutes. The supernatant was separated from the sediment and then was kept at 4 °C prior to analysis.

### Ultrasound-Assisted Extraction

Sonication of samples in water as extraction medium was done at low intensity level (<8.66 W/cm<sup>2</sup>) and duty cycle of 10, 20 and 40% by using an ultrasonic processor Q700 (max. power: 700 watts, output frequency: 20kHz) with a standard ½” horn made up of titanium alloy that had a tip diameter of 13 mm and approximately 136 mm in length from QSonica, Newtown, U.S.A. The 13 mm diameter tip of the sonotrode is inserted at half height of the extraction mixture of sample and water. The ultrasound amplitude was fixed at 1.00 A, which is the lowest amplitude to induce the ultrasound power of 11 W and power intensity of 8.66 W/cm<sup>2</sup>. A hotplate with thermometer probe was used to control the extraction temperature at 50.0, 70.0 and 100.0°C. The extraction was carried out for 8 hours and the extract was sampled for every hour. The crude extracts were centrifuged and analysed by using HPLC.

### Preparation of Calibration Curve

100 ppm of Vitexin stock solution was prepared and diluted in ultrapure water (Merck Millipore, USA). A calibrating curve of Vitexin (ranging between 0.01 – 0.08 mg/ml) was prepared by diluting stock solution with the ultrapure water.

### Chemicals

Standard of Vitexin (ChemFaces - CFN98601) was in ≥98% purity purchased from Wuhan ChemFaces Biochemical CO., Ltd. (Wuhan, Hubei, PRC). HPLC grade of methanol, formic acid (98-100%) and acetonitrile were purchased from Merck (Darmstadt, Germany).

### HPLC Analysis

The HPLC (Agilent, CA, USA) consisted of a computer-controlled system with G1379A Degasser, G1311A QuatPump and G1321A fluorescence detector. A reverse-phase Phenomenex (Torrance, CA, USA) column (Synergi, C<sub>18</sub>, 4µ, 250 X 4.60 mm i.d.) was used. Data acquisition was performed by Chemstation A.08.03. Some HPLC methods were available in the literature for the



determination of vitexin compound (Choo *et al.* 2012; Abdullah *et al.* 2009; Yang *et al.* 2009). In effort to find the most preferable method, some modifications have been done to the existing methods. The last trial has been changed its elution profile slightly to get better resolution. The column temperature was maintained at 25°C. Isocratic HPLC elution was used that consist of [A] 1.0% formic acid, water and [B] methanol. The mobile phases were filtered through a 0.2 µm nylon membrane and degassed prior to use. The flow rate was set at 1.0 mL/min. Fluorescence detection was conducted at 335 nm wavelength.

### Statistical Analysis

All experiments and analyses of samples were run in triplicates. Experimental results were expressed as means ± standard deviation errors. Statistical analysis was performed and the results obtained were analysed using one-way analysis of variance (ANOVA) for mean differences among the samples. Differences were considered to statistically significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Calibration Curve of Vitexin

The calibration curves obtained by plotting peak area of a series of analyses versus Vitexin concentration (ranging between 0.01 – 0.08 mg/ml) gave the linear regression equation with a determination coefficient ( $R^2$ ) of 0.9999 (Figure-1). The Vitexin content in *F. deltoidea* leaves extracts were calculated by using the calibration equation of reference compounds. Typical chromatograms obtained for *F. deltoidea* reference compounds and sample extract from aqueous and ultrasonic-assisted extraction are given in Figure-2. The peak corresponding to Vitexin in the extracts were assigned by comparing its retention time with that of reference compound.

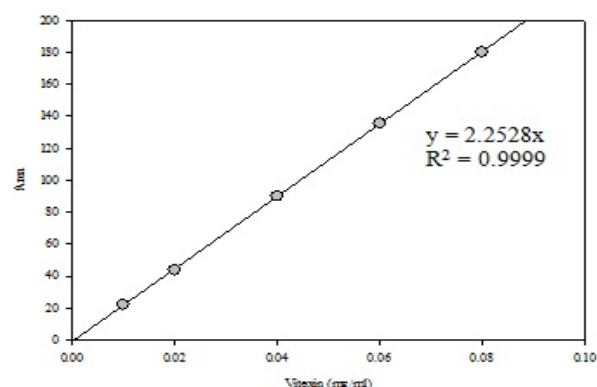


Figure-1. Calibration for Vitexin.

### Effect of Ultrasound and Temperature

Significant differences ( $p < 0.05$ ) were observed among the temperatures tested. The yield of Vitexin was expressed in actual percentage weight and the data were presented by a plotted graph of Vitexin value (%w/w)

against extraction period (hours). The effects of ultrasound can be measured by the data representing the different duty cycles. At 50 °C, the Vitexin values in UAE are much higher compared to the Vitexin values in AE. Different duty cycles show different yield of Vitexin. As shown in Figure-3 (a), the highest yield of Vitexin was recorded from UAE with duty cycle 10% at 4 hours of extraction. The Vitexin yield are also much higher in UAE than AE at 70 °C, but the highest value is recorded from UAE with 10% duty cycle at 5 hours of extraction, as shown in Figure-3 (b). However, at 100 °C, the highest Vitexin value was recorded from UAE with duty cycle 40% at 4 hours extraction.

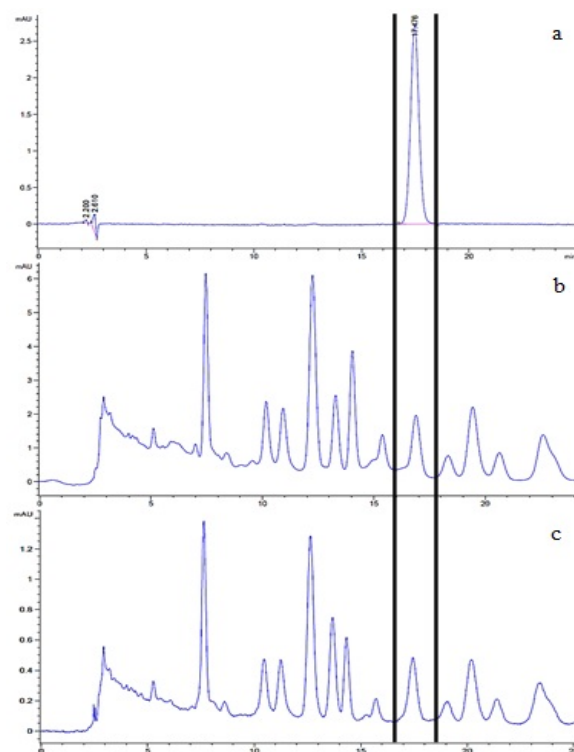
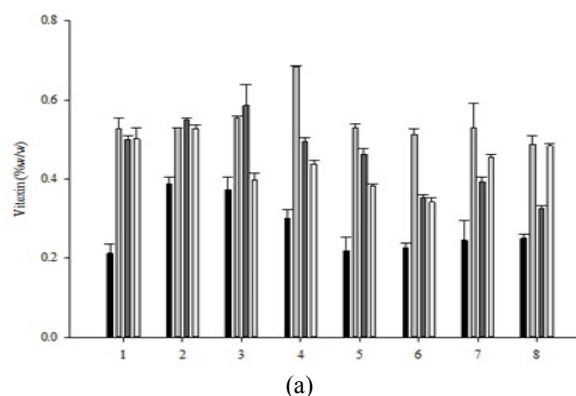
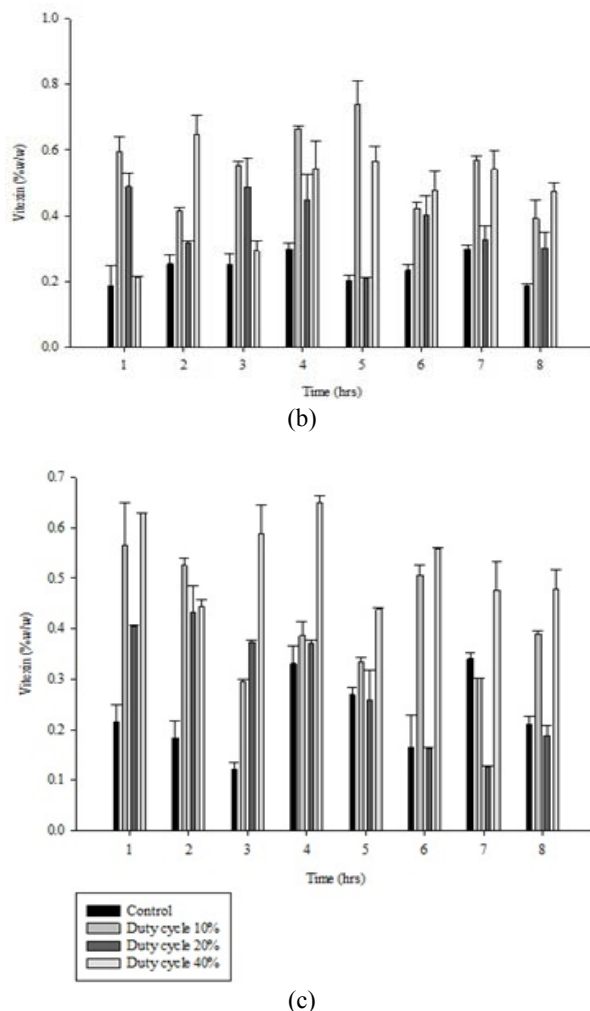


Figure-2. A sample HPLC chromatogram of *Ficus deltoidea* extracts: (a) mix reference standard of Vitexin, (b) sample from conventional extraction, (c) sample from ultrasonic-assisted extraction.





**Figure-3.** The effects of ultrasound and temperature on the extraction of Vitexin (ultrasound output power: 11 W; ultrasound intensity: 8.66 W/cm<sup>2</sup>; duty cycles of 10, 20 and 40% at: (a) 50, (b) 70, and (c) 100 °C.

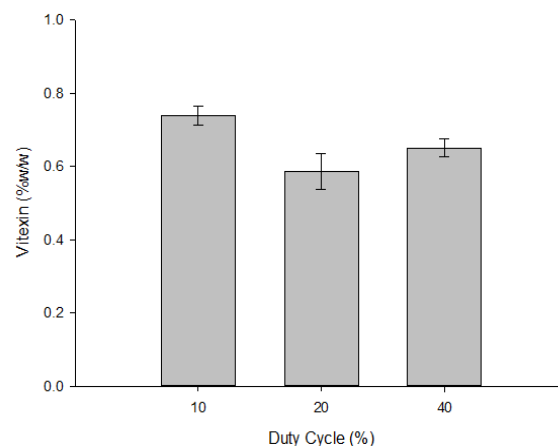
Among these three temperatures (50, 70 and 100 °C), the amount of extracted Vitexin in both AE and UAE shows the highest value at 50 °C. The value then decreased by 14.19% and 3.98% from 50 to 70 °C in AE and UAE respectively and 4.11% and 11.48% from 70 to 100 °C in AE and UAE respectively. The value of extracted Vitexin decreased as the temperature increase. In UAE, few cavitation bubbles were produced as a result of high acoustic cavitation threshold. The cell tissue disruption was enhanced by the relative greater force that caused by these bubbles explosion. However, at higher temperature (boiling temperature), more bubbles were created as a result of high vapour pressure, causing them to collapsed with low intensity due to a smaller pressure difference between inside and outside the bubbles (Hromadkova *et al.* 1999).

The bubbles may also be easily collapsed at higher temperature thus reducing the intensity of the mass

transfer enhancement. This also can be a possible explanation for the low Vitexin value in UAE compared to the value in AE at 100 °C. Based on these findings, 50 °C is the best temperature for the extraction of Vitexin in UAE. In terms of comparison between the two methods, the value of Vitexin has been improved in UAE compared to AE by 1.937-fold increase.

#### Effect of Duty Cycle

The effect of duty cycle of ultrasound on the extraction of Vitexin from *F.deltoidea* leaves is shown in Figure-4. At a duty cycle of 10% in combination with a power intensity of 8.66 W/cm<sup>2</sup>, the average value of Vitexin was nearly 1.26 and 1.135-fold of the duty cycle 20% and 40%. The most significant reason to this is that, a short irradiation pulse (i.e. 10% duty cycle) applied at 11 W irradiation power was most effective in producing Vitexin from *F.deltoidea* leaves. On the contrary, sonication at higher duty cycles of 20% and 40% was less effective due to the damage of compound by the prolonged sonication (Sulaiman *et al.* 2011).



**Figure-4.** The effect of duty cycle on the extraction of Vitexin [the error bar indicates the confidence interval at a confidence level of 95% (n=3)].

#### CONCLUSIONS

The potential of ultrasound-assisted technique was successfully explored and reported in this paper. Ultrasound can break the cell wall in cellulosic plant material, especially of *Ficus deltoidea* and this is yet to be fully exploited. It is hoping that the establishment of ultrasonic-assisted method could be used for the phyto-pharmaceutical and nutraceutical extraction industry. By this technique, it was proved that ultrasonically assisted extraction was a versatile technique that can be used both on a small and large scale. As one of the advanced extraction device, ultrasound can be applied not only to improve the quality and safety of extracted products, but offers the potential for developing new products with unique functionalities as well.



## REFERENCES

- [1] Abdul Hamid M., Oh M. J., Ngadiran S., Seo Y. K., Sarmidi M. R. and Park C. S. (2011). 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.*, 303, pp.161--170.
- [2] Abdullah Z., Hussain K., Ismail Z. and Ali R. M. (2009). Anti-inflammatory activity of standardized extracts of leaves of three varieties of Ficus deltoidea. *International Journal of Pharmaceutical and Clinical Research*, 1(3), pp.100--105.
- [3] Bunawan H., Amin N. M., Bunawan S. N., Baharum S. N. and Noor N. M. (2014). Ficus deloidea jack: A review on its phytochemical and pharmacological importance. *Evidence-Based Complementary and Alternative Medicine*, 2014, pp.1--9.
- [4] 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  $\alpha$ -glucosidase inhibition. *Journal of Ethnopharmacology*, 142, pp.776--781.
- [5] Dzolin S., Syed Aris S. R., Ahmad R. and Mat Zain M. (2010). Radical scavenging and neurotoxicity of four varieties of Ficus deltoidea. *International Conference on Science and Social Research*, Kuala Lumpur. 978-1-4244-8986-2.
- [6] Farsi E., Shafaei A., Hor S. Y., Ahamed M. K., Fei M., Yam Attitalla I. H., Asmawi M. Z. and Ismail Z. (2011). Correlation between enzymes inhibitory effects and antioxidant activities of standardized fractions of methanolic extract obtained from Ficus deltoidea leaves. *African journal of Biotechnology*, 10(67), pp.5184--5194.
- [7] Hadijah H., Normah A., Ahmad Tarmizi S. and Aida M. (2007). Cholesterol lowering effect of Mas cotek tea in hypercholesterolemic rats. *The 2<sup>nd</sup> International Conference of East-West Perspective of Functional Food Science*. 5-7 Nov. 2007, Kuala Lumpur. Organizer: ILSI.
- [8] Hakiman M. and Maziah M. (2009). Non enzymatic and enzymatic antioxidant activities in aqueous extract of different Ficus deltoidea accessions. *Journal of Medicinal Plants Research*, 3(3), pp.120--131.
- [9] Hromadkova Z., Kovacikova J. and Ebringerova A. (1999). Study of the classical and ultrasound-assisted extraction of the corn cob xylan. *Ind. Crops Prod*, 9, pp.101--109.
- [10] Kamal R. J., Wadood A. W. M. R. and Silva K. D. R. (2008). Total antioxidant capacity of selected grades of black tea grown in different geographical elevations in Sri Lanka. *Tropical Agricultural Research*, 20, pp.155--161.
- [11] 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 Fundamental Science*, 37(2), pp.195--201.
- [12] Ming P., Kuan W. S., Ling Y. X., Renyou X., Yucheng H. and Huijie L. (2013). Process study on the ultrasonic assisted enzymatic extraction of flavonoids from shepherd's purse. *Advanced Materials Research*, pp.634--638: 1281, 1286.
- [13] Misbah H., Abdul Aziz A. and Aminudin N. (2013). Antidiabetic and antioxidant properties of Ficus deltoidea fruits extracts and fractions. *BMC Complementary and Alternative Medicine*. 13, pp.118.
- [14] Omar M. H., Mullen W. and Crozier A. (2011). Identification of proanthocyanidins dimers and trimers, flavone C-glycosides, and antioxidants in Ficus deltoidea, a Malaysian herbal tea. *Journal of Agricultural and Food Chemistry*, 59, pp.1333--1369.
- [15] Ruzaina S., Mustafa S., Ahmat N., Jaafar F. M. and Ahmad R. (2009). Phenolic content and antioxidant activity of fruits of Ficus deltoidea var. angustifolia sp. *The Malaysian Journal of Analytical Sciences*, 13, pp.146--150.
- [16] Sulaiman A. Z., Ajit A., Yunus R. M. and Chisti Yusuf. (2011). Ultrasound-assisted fermentation enhances bioethanol productivity. *Biochemical Engineering Journal*, 54, pp.141--150.
- [17] Sulaiman M. R., Hussain M. K., Zakaria Z. A., Somchit M. N., Moin S., Mohamad A. S. and Israf D. A. (2008). Evaluation of the antinociceptive activity of Ficus deltoidea aqueous extract. *Fitoterapia*, 79, pp.557--561.
- [18] Wahid S., Mahmud T. M. M., Maziah M., Yahya A. and Rahim M. Ab. (2010). Total phenolics content and antioxidant activity of hot water extracts from dried Ficus deltoidea leaves. *Journal of Agriculture and Fundamental Science*, 38(1), pp.115--122.
- [19] Woon S. M., Seng Y. W., Ling A. P. K., Chye S. M. and Koh R. Y. (2013). Anti-adipogenic effects of Ficus deltoidea var. deltoidea and var. angustifolia on 3T3-L1 adipocytes. *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)*. ISSN 1673-1581.



- [20] Xiao Y. M., Wu Q., Cai Y. and Lin X. F. (2005). Ultrasound-accelerated enzymatic synthesis of sugar esters in nonaqueous solvents. *Carbohydrate Research*, 340, pp.2097--2103.
- [21] Yang B., Liu X. and Gao Y. (2009). Extraction optimization of bioactive compounds (crocin, geniposide and total phenolic compounds) from Gardenia (*Gardenia jasminoides* Ellis) fruits with response surface methodology. *Innovative Food Science and Emerging Technologies*, 10, pp.610--615.
- [22] Zhang Z. S., Wang L. J., Li D., Jiao S. S., Chen X. D. and Mao Z. H. (2008). Ultrasound-assisted extraction of oil from flaxseed. *Separation and Purification Technology*, 62, pp.192--198.