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ANTIOXIDANT AND ANTI-CANCER ACTVITY OF STANDARDIZED EXTRACTS OF THREE VARIETIES OF *Ficus deltoidea*'s LEAVES

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Graphical abstract

F. deltoidea var. *angustifolia*

F. deltoidea var. *deltoidea*

F. deltoidea var. *kunstleri*

Abstract

The present study was designed to evaluate the antioxidant and anti-cancer activities of aqueous extracts of three varieties of *Ficus deltoidea* (var. *angustifolia*, var. *deltoidea*, var. *kunstleri*) on prostate cancer DU145 cell line. The results showed that, *F. deltoidea* var. *kunstleri* contained the highest total phenolic (44.7 ± 0.022 mg GAE/10 g sample) and flavonoid contents (23.1 ± 0.005 mg CE/10 g sample) as well as the highest radical scavenging activity (IC_{50} 0.039 mg/mL) followed by *F. deltoidea* var. *deltoidea* and *F. deltoidea* var. *angustifolia*. Similarly, based on anti-cancer activities, *F. deltoidea* var. *kunstleri* demonstrated the lowest IC_{50} value (93.11 μg/mL) followed by *F. deltoidea* var. *deltoidea* (204.17 μg/mL) and *F. deltoidea* var. *angustifolia* (429.54 μg/mL). Other than that, vitexin which is a bioactive marker was appeared to be the highest in *F. deltoidea* var. *kunstleri* compared to others. Hence, the results suggested that there might be an association between antioxidant activities and bioactive markers against prostate cancer cell line (DU145).

Keywords: *F. deltoidea*, antioxidant, anti-cancer

Abstrak

Kajian ini telah direka bentuk untuk menilai aktiviti antioksidan dan anti-kanser ekstrak akueus daripada tiga variasi *Ficus deltoidea* (var. *angustifolia*, var. *deltoidea*, var. *kunstleri*) terhadap kanser prostat. Hasilnya menunjukkan bahawa *F. deltoidea* var. *kunstleri* mempunyai jumlah fenolik (44.7 ± 0.022), kandungan flavonoid (23.1 ± 0.005 mg CE/10 g sampel) dan aktiviti anti-radikal (IC_{50} 0.039 mg/mL) tertinggi diikuti oleh *F. deltoidea* var. *deltoidea* dan *F. deltoidea* var. *angustifolia*. *Begitu juga,* berdasarkan aktiviti anti-kanser *F. deltoidea* var. *kunstleri* menunjukkan nilai IC_{50} (93.11 μg/mL) terendah diikuti dengan *F. deltoidea* var. *deltoidea* (204.17 μg/mL) dan *F. deltoidea* var. *angustifolia* (429.54 μg/mL). Selain itu, vitexin iaitu penanda bioaktif paling banyak didapati dalam *F. deltoidea* var. *kunstleri* berbanding yang lain. Oleh itu, hasil kajian mencadangkan terdapat kemungkinan kaitan antara aktiviti antioksidan dan penanda bioaktif terhadap sel kanser prostat.

Kata kunci: *F. deltoidea*, antioksidan, anti-kanser

1.0 INTRODUCTION

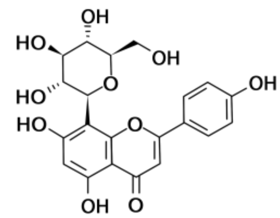
Prostate cancer has becoming the most leading cause of death among men over the world after bronchus [1]. Every year, the prevalence and incidence rate of prostate cancer have increased rapidly especially in Western countries. It is notable that 1.1 million of men were having prostate cancer [2] and 25 % of men were diagnosed to have prostate cancer in United Kingdom [3]. Previously, prostate cancer was commonly being associated with hormone androgen dependence but it not happen at present as many other factors associated and remain unclear [4-5].

An extensive research and studies are building in order to come across the best and an appropriate explanation to reduce the cases. It includes identification of biomarkers of the cancer [6], identification of gene mutations as well as the role of arachidonic acid metabolism through cyclooxygenase [7-8] lipoxygenase [9] and P450 epoxygenase pathways [10-12]. In such cases, the treatment might be different from others.

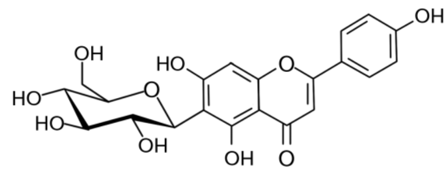
Many studies had been conducted by researchers and proved that plants-containing phenolic compounds can be used in the prevention and treatment of cancer [13] by regulation of growth factor – receptor interactions and cell signaling cascade [14]. Other than that, it have been recognized that the abundance of bioactive markers like vitexin (**1**) and isovitexin (**2**) presence in *Ficus deltoidea* may contribute to the pharmacological effects [15].

Vitexin (**1**) is an apigenin-8-C-D-glucopyranoside, a natural flavonoid which is causing apoptosis and suppressing activity of tumor growth interconnection to the inhibition of cancer activities [16]. Indeed. It also responsible to cause anti-metastatic and apoptotic effect *via* p53 in human oral cancer cells. Thus, it was recommended that the presence of (**1**) as a bioactive marker may contribute to the effect of cytotoxicity against prostate cancer.

Ficus deltoidea comes from the family of Moraceae, genus *Ficus*. In Malaysia, it is known as Mas Cotek or sempit-sempit however for Indonesian, it is called as Tabat barito. Other than *F. deltoidea*, the vernacular names are Delta fig, Fig shrub, and Mistletoe fig. It is well distributed throughout the Southeast Asia and can also be found in Africa. To date, there are about 13 varieties of *F. deltoidea* which are based on morphological of leaves and figs [17]. *F. deltoidea* has been proven to have many pharmacological effects on human body like antimicrobial [18], antinociceptive [19], antioxidant [20-21] as well as anti-inflammatory [22-24]. A study has proved that *F. deltoidea* has anti-cancer effect against ovarian carcinoma [25] and leukemic HL-60 cell line [26]. To date, no study has been done on the relationship between antioxidant and anti-cancer effect of prostate cancer by *F. deltoidea*.



(1)



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2.0 EXPERIMENTAL

2.1 Plant Materials

The leaves of three varieties of *F. deltoidea* i.e *F. deltoidea* var. *angustifolia* (FD 1), *F. deltoidea* var. *deltoidea* (FD 2) and *F. deltoidea* var. *kunstleri* (FD 3) were selected in this study. They were purchased from Moro Seri Utama Enterprise, Malaysia.

2.2 Extraction

The ground leaves (7 g) of each three varieties were boiled in distilled water (300 mL) at 90°C for 1 hour and 50 minutes. After that, the aqueous extracts were filtered and concentrated using rotary evaporator at 40°C, freeze-dried and stored at -20°C until further use. The yield of the extracts was calculated based on dry weight basis using an equation as below. Extraction gave: FD 1; 27.8 %, FD 2; 37.6 %, and FD 3; 24.6 %.

$$\text{Yield (\%)} = \frac{\text{weight after freeze dried}}{\text{weight of fresh sample}} \times 100\ \% \qquad [1]$$

2.3 Cell Cultures

Human Prostate cancer cell line, DU145 was obtained from Faculty of Chemical Engineering, Universiti Teknologi Malaysia, Malaysia. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) media supplemented with 10 % fetal bovine serum and 1 % penicillin-streptomycin in static 75 cm^2 (USA). The cells were incubated in a humidified atmosphere with 5 % CO_2 T-Flask (GIBCO), at 37°C.

2.4 High Performance Liquid Chromatography (HPLC)

Vitexin (**1**) and isovitexin (**2**) were analyzed using a Waters HPLC system (Milford, MA, USA) consisting of a pump and system controller (Model 2695) and photo-diode array detector (Model 966). This separation was done by a reversed phase column (4.6 × 150 mm, 4 µm; Phenomenex, Torrance, CA, USA). The Separation was achieved by flow rate of 1 mL/min with 1 % of formic acid (66 %) / methanol (34 %), in an isocratic programme. The samples (20 µL) were introduced into the column using an autosampler. The detection was monitored at 330 nm and data were integrated by Empower 2 software (Waters) (Milford, MA, USA).

2.5 Preparations of the Standard Solution and Samples

The stock solution of (**1**) (1000 µg/mL) and (**2**) (500 µg/mL) were prepared by dissolving 2 mg and 1 mg of standards in methanol (2 mL), respectively. The calibration curve was made by mixing the standards in order to get concentration of 1 µg/mL – 100 µg/mL. The aqueous extracts of three varieties (1 mg each) were dissolved in 2 mL of distilled water followed by filtration using syringe filter 0.45 µm. All samples were kept at -20°C prior to further use.

2.6 MTT Assay

The cells were placed in a 96-well-plate with 1 x 10^4 cells/well of concentration. After 48 hours of incubation, the culture medium was removed and replaced with medium without serum for starvation. Then, the cells were exposed to various concentrations of aqueous extracts range from 2.4 mg/mL - 0.007 mg/mL for 48 hours. Subsequently, MTT reagent (5 mg/mL in sterile PBS) was added directly to the wells. Cells were then returned to the incubator for another 4 hours. After that, the medium and the MTT reagent mixture were gently removed and 200 µL DMSO was added for 5 minutes to each well. The absorbance was measured at 570 nm using an ELISA plate reader. All samples were assayed in triplicate. The growth of inhibition was determined by the following equation:

$$\% \text{ Cell viability} = \frac{\text{Sample Optical Density}}{\text{Control Optical Density}} \times 100\ \% \quad [2]$$

2.7 Determination of Total Phenolic Contents (TPC)

Total phenolic content (TPC) of three varieties of *F. deltoidea* were carried out using Folin-Ciocalteu assay and gallic acid was used as standard (ranging from 0.02 - 0.10 mg/mL). In brief, 5 mL of Folin-Ciocalteu reagent was added into the sample or standard. Then, they were mixed for five minutes. After that, 4 mL of Na_2CO_3 solution was added and left for 60 minutes. The absorbance was read at 760 nm by using UV/VIS spectrophotometer. A gram of garlic acid equivalents (GAE) per 10 g of extract was used as results of total phenolic content.

2.8 Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) tests of three varieties of *F. deltoidea* were performed based on the method published by Atanassova *et al*, [27] with slight modification. 1 mL of sample or standard stock solution was added into a 10 mL volumetric flask followed by 4 mL of distilled water. Afterwards, the solution was mixed up with 0.3 mL of $NaNO_2$.. After five minutes, 0.3 mL of $AlCl_3$ was added into the volumetric flask and the mixtures were left for another six minutes. Next, 2 mL of NaOH was added followed by distilled water to make up to a 10 mL of volumetric flask. The analyses were then performed in triplicate and the standard curve with serial catechin solution (0.05, 0.10, 0.25 and 0.50 mg/mL) was used for calibration. The absorbance was read at 510 nm and the results were expressed as a gram of catechin equivalents (CE) per 10 g of extract.

2.9 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

The stock solutions of the crude extracts were prepared in 1 mg/mL in distilled water to obtain various concentrations (0.6, 0.25, 0.13, 0.06, 0.03 mg/mL). Next, 2 mL of DPPH solution was added to 1 mL of each sample and the mixture were placed in the dark for 30 minutes at room temperature. Butylated hydroxyl anisole (BHA) and Vitamin C were used as reference standards. The absorbance was measured at 517 nm and the activity of radical scavenging was calculated using the following formula:

Inhibition of DPPH scavenging activity (%) =

$$\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\% \quad [3]$$

2.10 Statistical Analysis

All analyses were done in triplicate and the data were presented as means ± standard deviation. The ANOVA (one-way) was used to analyze the data and $p < 0.05$ was set as the limits of significance. The IC_{50} were obtained through GraphPad Prism.

3.0 RESULTS AND DISCUSSION

3.1 HPLC Analysis of Different Varieties of *F. deltoidea*

HPLC analysis was used to quantify and identify the individual compound in samples by comparing the retention times of standard with samples (Figure 1).

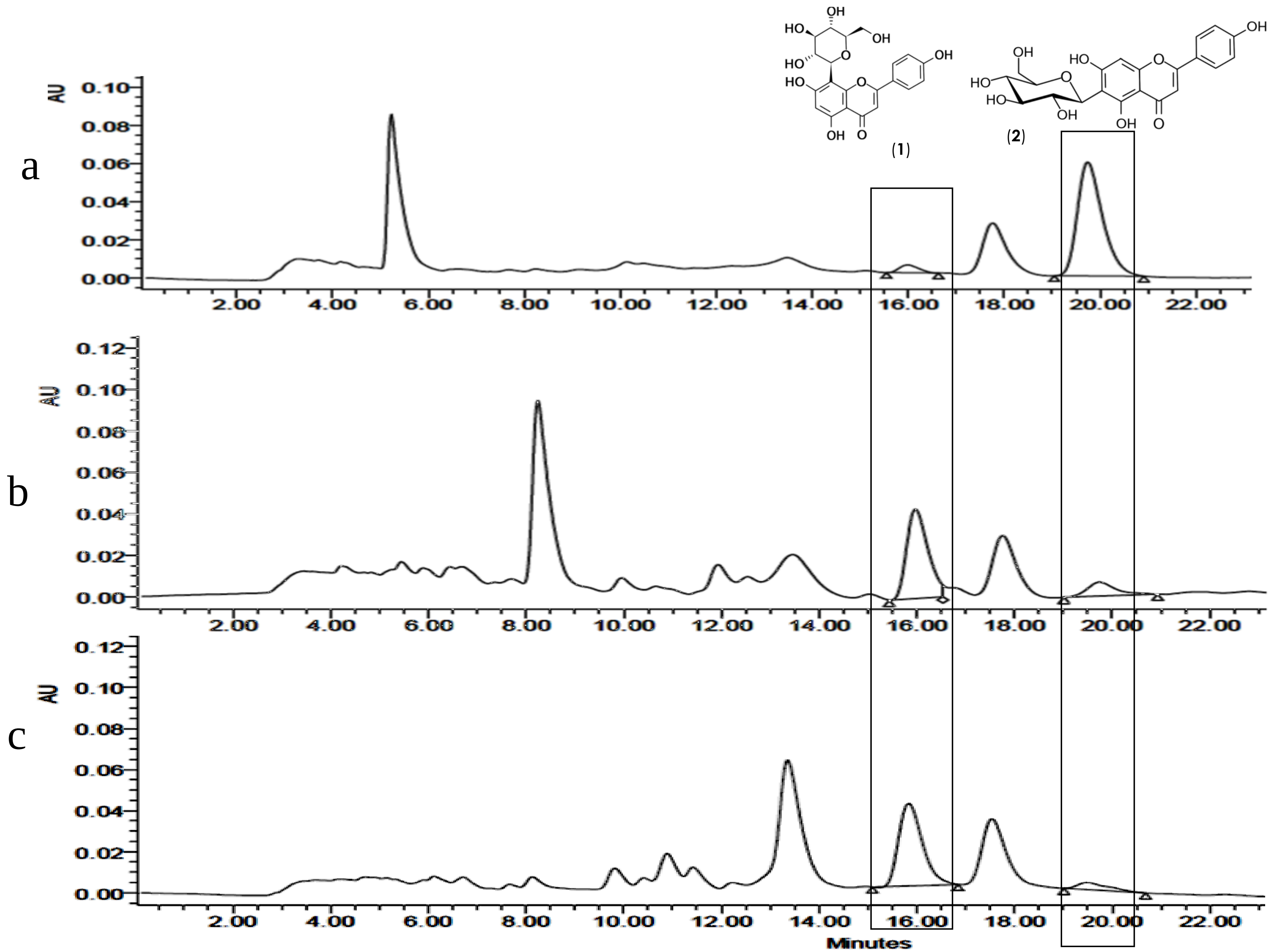

Figure 1 Chromatograms of (**1**) and (**2**) of leaves of *F. deltoidea*; a (FD 1), b (FD 2), c (FD 3) detected by HPLC at wavelength 330 nm

The concentrations of the samples were calculated based on the linearity of calibration curves of (**1**) and (**2**) (1– 100 μg/mL) in which X is the concentration of the compound while Y is a peak area. Compound (**1**) showed good linearity with regression equation Y = 22955x + 48627, R^2 0.995, as well as (**2**) with regression equation Y = 33932 x + 46459, R^2 = 0.996).

The results demonstrated that *F. deltoidea*. var. *kunstleri* (FD 3) contained the highest of (**1**) followed by var. *deltoidea* (FD 2) and var. *angustifolia* (FD 1). Nevertheless, (**2**) was highest in var. *angustifolia* (FD 1) compared to others. The results were similar to the study done by Azemin *et al*. [2] in which they found that (**2**) was highest in *F deltoidea* var. *angustifolia* (FD 1).

Table 1 Quantitative analysis of vitexin (**1**) and isovitexin (**2**) (mg/g) in three different extracts of *F. deltoidea* ± STD

Extract	Vitexin (1)(mg/g)	Isovitexin (2)(mg/g)
FD 1	0.3 ± 0.07	5.3 ± 0.12
FD 2	2.8 ± 0.18	0.3 ± 0.09
FD 3	5.1 ± 0.09	0.24 ± 0.056

FD 1 = *F. deltoidea* var. *angustifolia*, FD 2 = *F. deltoidea* var. *deltoidea*, FD 3 = *F. deltoidea* var. *kunstleri*

3.2 Antioxidant Activity of Different Varieties of *F. deltoidea*

The results showed that, FD 3 was rich with phenolic content followed by FD 2 and FD 1. Similarly, FD 3 exhibited the highest total flavonoid content with value of 23.1 mg CE/10 g sample followed by FD 2 and FD 1 (Table 2). Even though the species used in this study were similar, but the total phenolic and flavonoids as well as radical scavenging activity may vary upon the varieties used. Besides, the metabolomic of plants can also affect the activity tested [28-29]. Based on DPPH assay, FD 3 demonstrated the most potent scavenging activity with IC_{50} value of 0.03 mg/mL followed by FD 2 (0.054 mg/mL) and FD 1 (0.14 mg/mL) (Figure 2).

3.3 Anticancer Activity

The percentage of viability cell of DU 145 cell line with different concentrations (2.4 mg/mL - 0.007 mg/mL) of three varieties of *F. deltoidea* after 48 hours incubation are presented in Figure 3. The results were determined by the decolourisation of soluble MTT into purple insoluble formazan which later detected by ELISA reader at 517 nm. The more viable of DU145 indicated the lower inhibition towards prostate cancer. It was showed that all varieties used posses' anticancer activity against DU 145 cell line. However, the maximum DU 145 cell line inhibition was observed in *F. deltoidea* var. *kunstleri* (FD 3) where the IC_{50} value was the lowest (93.11 μg/mL), followed by. *F. deltoidea* var. *deltoidea* (FD 2) (204.17 μg/mL) and *F. deltoidea* var. *angustifolia* (FD 1) (429.54 μg/mL) (Figure 3 and 4). Previous study [25] also had obtained the IC_{50} of 224.39 μg/mL and 143.03 μg/mL for aqueous and ethanolic extracts of *F. deltoidea* (not mentioned varieties), respectively on Human Ovarion Carcinoma Cell Line.

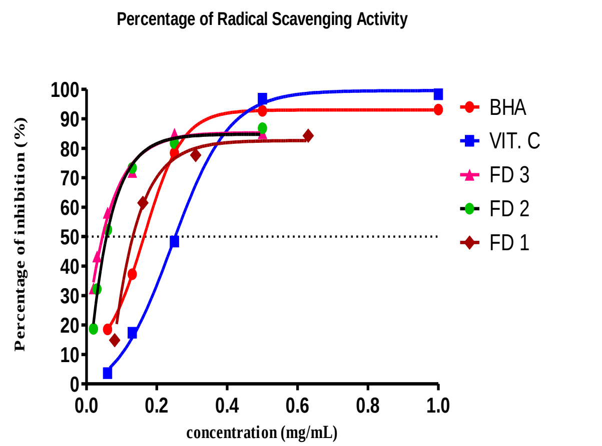

Figure 2 The IC_{50} values found in aqueous extracts of three varieties of *F. deltoidea*

Table 2 Total phenolic content and flavonoid content of *F. deltoidea*

Extract	Total Flavonoid (mg CE/10 g sample)	Total phenolic (mg GAE/10 g sample)
FD 1	2.7 ± 0.006	17.6 ± 0.017
FD 2	20.17± 0.012	31.8 ± 0.009
FD 3	23.1 ± 0.005	44.7 ± 0.022

Values are mean of three replicate determination ± standard deviations; Weight of dried leaves was 6.89 g

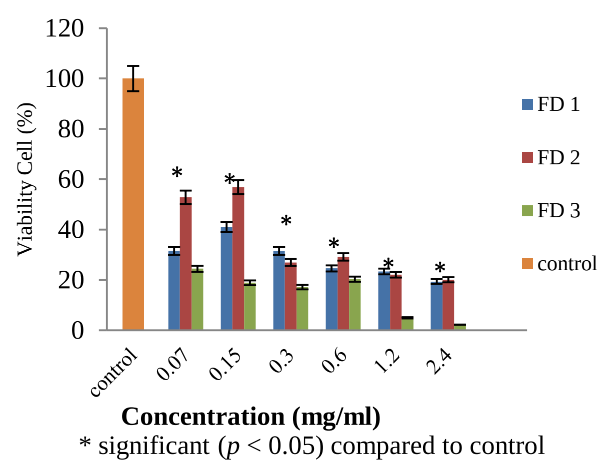

Figure 3 Effect of various concentrations of aqueous extracts of *F. deltoidea* on the cell viability in MTT assay after 48 hours treatment. * Significant ($p < 0.05$) compared to control

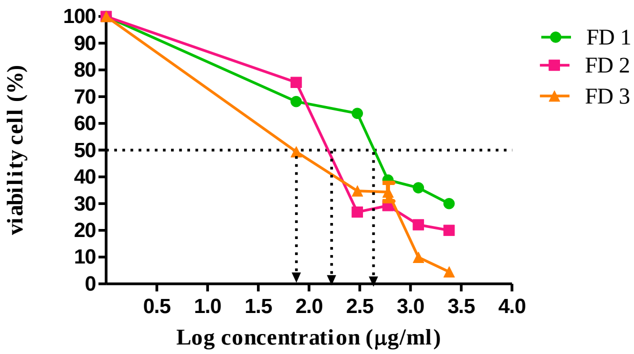

Figure 4 The cytotoxicity of the aqueous extracts of three varieties of *F. deltoidea* determined by IC_{50} values

4.0 CONCLUSION

The leaves of *F. deltoidea* have been proven to exert many pharmacological effects as they encompass abundant of compounds like vitexin (**1**) and isovitexin (**2**). The antioxidant activity found in three varieties of the species was believed to involve in anti-cancer activity of prostate cancer. Adefegha and Oboh have confirmed that the occurrence of cancer is due to the presence of unstable molecules in the body [30]. When body contains of high free radical, it will give harm and cause damage to the cellular or genomic in the prostate cell and later lead to the development of the cancer. Previous studies related to antioxidant and phenolic content on plant have been discussed throughout the years. Based on the findings, the phenolic compounds can inhibit the initiation, progression and spread of the cancer. As the cancer can be caused by a process of angiogenesis, the reduction of eicosonoid which is believed comes from anti-inflammatory effect of *F. deltoidea* can help to inhibit the development of blood vessels for the growth of tumors. Basically, plant with higher amounts of flavonoids may indicate the significance of medicinal properties as studies showed that plants rich with flavonoids may act as anticancer and antitumor agents [31-33]. Other than that, it also has been stated that antioxidant activities of phenolic compounds had been proven to have a connection on anticancer activities [34]. Thus, it can be recommended that *F. deltoidea* have potent natural antioxidant which beneficial to overcome and reduce the risk of prostate cancer.

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References

[1] Harborne, J. 1973. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* New York: John Wiley and Son Inc.
[2] Azemin, A., Dharmaraj, S., Hamdan, M. R., Mat, N., Ismail, Z. *and Mohd, K. S. (2014). Discriminating Ficus deltoidea var. bornensis from Different Localities by HPTLC and FTIR Fingerprinting. Journal of Applied Pharmaceutical Science*. 4 (11): 069-075.
[3] Hwa, J. C., Jae, S. E., Bang, G. K., Sun, Y. K., Hoon, J. and Yunjo, S. 2006. Vitexin, an HIF-1α Inhibitor, Has Anti-metastatic Potential in PC12 Cells. *Molecules and Cells*. 22 (3): 291-299.
[4] Agarwal, N., Lorenzo, G.D., Sonpavde, G. and Bellmunt, J. 2014. New Agents for Prostate cancer. *Annals of Oncology*. 1-9.
[5] Alan, E. N., Rodney, T. J. and Colin, U. M. 1998. Non-steroidal Anti-inflammatory Drugs and Prostate Cancer Progression. *International Journal of Cancer*. 77: 511–515.
[6] William, E. G., David, G. B., Harry, B., Ossama, W. T., Dougles, M., Jeffrey, C. and Ronal, L. 2005. Biomarkers in Prostate Cancer. *American Association for Cancer Research 96th Annual Meeting*. 196-202.
[7] Wolfram, D., Christoph, R. and Axel, G. 2001. Cyclooxygenase-2: A Novel Target for Cancer Chemotherapy. *Journal of Cancer Research and Clinical Oncology*. 127: 411-417.
[8] Greenhough, A., Smartt, H. J. M., Moore, A. E., Roberts, H. R., Williams, A. C., Paraskeva, C. and Kaidi, A. 2009. The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis*. 30(3): 377-386.
[9] Qihan, D., Manish, P., Kieran, F. S., Garry, G. G., Pamela, J. R. and Paul, S. 2006. Oncogenic action of phospholipase A_2 in prostate cancer. *Cancer Letters*. 240: 9-16.
[10] Attiga, F. A., Fernandez, P. M., Weeraratna, A. T., Manyak, M. J. and Patierno, S. R. 2000. Inhibitors of Prostaglandin Synthesis Inhibit Human Prostate Tumor Cell Invasiveness and Reduce the Release of Matrix Metalloproteinases. *Cancer Research*. 60(16): 4629-4637.
[11] Mushtaq Ahmad, Mir Ajab Khan, Sarfaraz Khan Marwat, Muhammad Zafar, Muhammad Aslam Khan, Tamoor Ul Hassan and Shazia Sultana. 2009. Useful Medicinal Flora Enlisted in Holy Quran and Ahadith. *American-Eurasian Journal of Agricultural and Environmental Sciences.* 5(1): 126-140.
[12] Sahreen, S., Khan, M. R. and Khan, R. A. 2014. Effects of *Carissa opaca* fruits extracts on oxidative pulmonary damages and fibrosis in rats. BMC Complementary and Alternative Medicine. 14(1): 1-9.
[13] Wahle, K. J., Brown, I., Rotondo, D. and Heys, S. 2010. Plant Phenolics in the Prevention and Treatment of Cancer. In M. Giardi, G. Rea and B. Berra (Eds.). *Bio-Farms for Nutraceuticals*. 698: 36-51.
[14] 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: 557-561.
[15] Islam, S., Akhtar, M., Parvez, S., Alam, J. and Alam, F. M. 2013. Antitumor and antibacterial activity of a crude methanol leaf extract of *Vitex negundo* L. *Archives of Biological Sciences.* 65(1): 229-238.
[16] Zhou, Y., Liu, Y._E., Cao, J., Zeng, G., Shen, C., Li, Y., Zhou, M., Chen, Y., Pu, W., Potters, L. and Shi, Y._E. 2009. Vitexins, nature-derived lignan compounds, induce apoptosis and suppress tumor growth. *Clinical Cancer Research*. 15(16):5161-5169.
[17] Corner, E. J. H. 1969. The complex of *Ficus deltoidea*; A recent invasion of the Sunda Shelf. *Philosophical Transactions of the Royal Society of London*. 256: 281-317.

[18] Jain, N. K., Siddiqi, M. A. and Weisburger J. H. 2006. *Protective effects of tea on human health*. United Kingdom: Cromwell Press.

[19] Schulz, W. A., Burchardt, M. and Cronauer, M. V. 2003. Molecular Biology of Prostate Cancer. *Molecular Human Reproduction*. 9(8): 437-448.

[20] Nie, D., Che, M., Grignon, D., Tang, K. and Honn, K. 2002. Role of eicosanoids in prostate cancer progression. In M. Cher, A. R and Honn, K. (Eds.), *Prostate Cancer: New Horizons in Research and Treatment* (pp. 59-70). United State: Springer.

[21] Lee, S. W., Wendy, W., Julius, Y. F. S. and Desy Fitrya Syamsumir. 2011. Characterization of antioxidant, antimicrobial, anti cancer property and chemical composition of *Ficus deltoidea* Jack. Leaf extract. *Journal of Biologically Active Products from Nature*. 2231-1874.

[22] Zakaria, Z. A., Hussain, M. K., Mohamad, A. S., Abdullah, F. C. and Sulaiman, M. R. 2011. Anti-Inflammatory Activity of the Aqueous Extract of *Ficus deltoidea*. *Biological Research for Nursing*. 14(1): 90-97.

[23] Zunoliza, A., Khalid, H., Zhari, I. and Rasadah, M. A. 2009. Anti-inflammatory Activity of Standardised Extracts of Leaves of Three Varieties of *Ficus deltoidea. International Journal of Pharmaceutical and Clinical Research*. 1(3): 100-105.

[24] Yizhong, C., Qiong. L., Mei, S. and Harold, C. 2003. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*. 74: 2157–2184.

[25] Nor Azurah Mat Akhir, Lee Suan Chua, Fadzilah Adibah Abdul Majid and Mohamad Roji Sarmidi. (2011). Cytotoxicity of Aqueous and Ethanolic Extracts of *Ficus deltoidea* on Human Ovarian Carcinoma Cell Line. *British Journal of Medicine and Medical Research*. 1(4): 397-409.

[26] Norrizah, J. S, Norizan, A., Sharipah Ruzaina, S.A., Dzulsuhaimi, D. and Nurul Hidayah, M. S. 2012. Cytotoxicity Activity and Reproductive Profiles of Male Rats Treated with Methanolic Extracts of *Ficus deltoidea. Research Journal of Medicinal Plant*. 6: 197-202.

[27] Atanassova, M., Marinova, D. and Ribarova, F. 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *Journal of the University Chemical Technology and Metallurgy.* 40: 255-260.

[28] Jain, N. K., Siddiqi, M. A. and Weisburger J. H. 2006. *Protective effects of tea on human health*. United Kingdom: Cromwell Press.

[29] Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteau reagent. *Methods in Enzymology.* 299: 152-178.

[30] Adefegha, S. A. and Oboh. G., 2011. Cooking enhances the antioxidant properties of some tropical green leafy vegetables. *African Journal Biotechnology*.10(4): 632-639.

[31] Wahle, K. J., Brown, I., Rotondo, D. and Heys, S. 2010. Plant Phenolics in the Prevention and Treatment of Cancer. In M. Giardi, G. Rea and B. Berra (Eds.), *Bio-Farms for Nutraceuticals* (pp. 36-51). United State: Springer.

[32] International Agency for Research on Cancer. 2012. *Prostate Cancer Estimated Incidence, Mortality and Prevalence Worldwide in 2012*. Retrieved September, 6, 2014 from http://www.iarc.fr/

[33] Olayinka, O. O., Kareem, A. M., Ariyo, I. B., Omotugba, S. K. and Oyebanji, A. O. 2012. Antioxidant contents (Vitamin C) of raw and blenched different fresh vegetable samples. *Food and Nutrition Sciences*. 3: 18-21.

[34] Himmat, S. C. 2011. Prospective Study of Antioxidants, Its Mechanism and Potential Role in Cancer. *International Journal of Research in Pharmaceutical and Biomedical Science*. 2 (3): 888-894.