

PROSTATE CANCER CELL INHIBITION BY ETHANOLIC FRACTION OF *FICUS*  
*DELTOIDEA* VAR. *KUNSTLERI*

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## ABSTRACT

*Ficus deltoidea* or also known as 'Mas Cotek' belongs to the Moraceae family. It is widely used by practitioners for post-natal treatment, anti-diabetes and anti-hypertension. Study has documented that they were at least 13 different varieties of *F. deltoidea*. However, the present study only accounted for three varieties of *F. deltoidea* for the evaluation of their antioxidant and anti-cancer effects against prostate cancer cell. The varieties include *F. deltoidea* var. *angustifolia*, *F. deltoidea* var. *deltoidea* and *F. deltoidea* var. *kunstleri*. The study was divided into two parts whereby the best variety and the potent fraction were determined. The aqueous extract of *F. deltoidea* var. *kunstleri* was selected for fractionation using solid phase extraction as it demonstrated the highest content of phenolic, flavonoid, vitexin as well as lowest concentration to inhibit radical scavenging activity,  $IC_{50}$  0.043 mg/mL. Moreover, cytotoxicity study through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay also demonstrated that *F. deltoidea* var. *kunstleri* had the lowest  $IC_{50}$  of 93.11  $\mu$ g/mL. The fractionation was accomplished using mixture of different ratio of ethanol/water. Eluent 1 (EL 1) was fractionated using 20% of ethanol/water exhibited the highest polyphenol contents and the highest inhibition against prostate cancer at 100  $\mu$ g/mL (45.45%). The mechanism involved in the reduce cell viability exerted by *F. deltoidea* var. *kunstleri* and EL 1 was due to apoptosis and it was confirmed using flow cytometry. In the event of apoptosis, cells treated with *F. deltoidea* var. *kunstleri* and EL 1 undergoes morphological changes that comprised of cell condensation, fragmentation and necrosis. Moreover, the Fourier transform infrared spectroscopy - attenuated total reflectance analysis showed that EL 1 contained other chemical compounds that created a synergistic effect toward cells inhibition. Positive correlations were also established among antioxidant and anti-cancer activities of varieties and fractions. Therefore, these results suggested that antioxidant activity of *F. deltoidea* var. *kunstleri* and its fraction, EL 1 with the presence of polyphenols and vitexin can potentially be used as an anti-cancer agent especially for prostate cancer.

## ABSTRAK

*Ficus deltoidea* atau juga dikenali sebagai 'Mas Cotek' tergolong dalam keluarga Moraceae. Ia digunakan secara meluas oleh pengamal untuk rawatan selepas bersalin, anti-diabetes dan anti-hipertensi. Kajian telah mendokumenkan bahawa terdapat sekurang-kurangnya 13 variasi *F. deltoidea*. Walau bagaimanapun, kajian ini hanya mengambil tiga variasi *F. deltoidea* untuk penilaian kesan antioksidan dan anti-kanser terhadap sel kanser prostat. Variasi - variasi itu termasuk *F. deltoidea* var. *angustifolia*, *F. deltoidea* var. *deltoidea* dan *F. deltoidea* var. *kunstleri*. Kajian ini dibahagikan kepada dua bahagian di mana variasi yang terbaik dan pecahan paling kuat telah ditentukan. Ekstrak akueus *F. deltoidea* var. *kunstleri* telah dipilih untuk pemecahan menggunakan pengestrakan fasa pepejal kerana ia menunjukkan kandungan tertinggi fenolik, flavonoid, vitexin serta kepekatan terendah untuk menghalang aktiviti memerangkap radikal,  $IC_{50}$  0.043 mg/mL. Selain itu, kajian sitotoksik melalui assai 3-(4,5-dimetiltiazol-2-yl)-2,5-difeniltetrazolium bromida juga menunjukkan bahawa *F. deltoidea* var. *kunstleri* mempunyai  $IC_{50}$  terendah 93.11  $\mu$ g/mL. Pemecahan telah dicapai dengan menggunakan campuran nisbah yang berbeza dari etanol/air. Bahan pengelusi 1 (EL 1) yang terpecah menggunakan 20% daripada etanol/air mempamerkan kandungan polifenol tertinggi dan perencatan yang paling tinggi terhadap kanser prostat pada 100  $\mu$ g/mL (45.45%). Mekanisma yang terlibat dalam pengurangan sel oleh *F. deltoidea* var. *kunstleri* dan EL 1 adalah disebabkan oleh apoptosis dan ia disahkan menggunakan sitometri. Dalam keadaan apoptosis, sel-sel yang dirawat dengan *F. deltoidea* var. *kunstleri* dan EL 1 telah mengalami perubahan morfologi seperti pengecutan, pemecahan dan nekrosis. Tambahan pula, analisis spektroskopi inframerah transformasi Fourier – pantulan keseluruhan dikesilkan menunjukkan bahawa EL 1 mengandungi sebatian kimia yang tidak diketahui yang menghasilkan kesan sinergi ke arah perencatan sel. Korelasi positif juga telah terbukti antara aktiviti antioksidan dan anti-kanser. Oleh itu, keputusan ini mencadangkan bahawa aktiviti antioksidan *F. deltoidea* var. *kunstleri* dan pecahannya, EL 1 adalah disebabkan kehadiran polifenol dan vitexin yang berpotensi untuk digunakan sebagai agen anti-kanser terutama bagi kanser prostat.

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**LIST OF SYMBOLS**

|       |   |                        |
|-------|---|------------------------|
| mg    | - | Miligram               |
| g     | - | Gram                   |
| cm    | - | Centimeter             |
| L     | - | Liter                  |
| mL    | - | Mililiter              |
| ppm   | - | Part per million       |
| °C    | - | Degree celcius         |
| <     | - | <i>Less than</i>       |
| >     | - | <i>Greater than</i>    |
| $r$   | - | Correlation index      |
| $R^2$ | - | Linear regression      |
| RPM   | - | Revolutions per minute |
| %     | - | Percentage             |
| μg    | - | Microgram              |

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Background of the Study

Prostate cancer has become the leading cause of death among men over the world after bronchus (Henry *et al.*, 1999). Every year, the prevalence and incidence rate of prostate cancer has increased rapidly especially in the Western countries. In 2012, it has been estimated that 1.1 million of men have prostate cancer (International Agency for Research on Cancer, 2012) and out of 25 in 100 men were diagnosed to have prostate cancer in the United Kingdom (Cancer Research UK, 2014). Moreover, there are many risk factors associated with prostate cancer. It might due to the life style, strong family history, diet and environment (William *et al.*, 2005; Henry *et al.*, 1999). The incidence and mortality of the prostate cancer is very closely related to age.

In the past, prostate cancer is commonly being associated with hormone androgen dependence (Agarwal *et al.*, 2014) but it does not happen at present as many other factors were associated and remain unclear (Alan *et al.*, 1998). Due to that factor, DU145 cell line prostate cancer was selected in this study. The cell line corresponds to hormone independent cell line and a type of early prostate cancer cell.

Qihan *et al.* (2006) also emphasized that prostate cancer could be due to the high production of eicosanoids which were responsible to promote angiogenesis and cell invasion. An extensive research and studies were built in order to discover the best and an appropriate explanation to reduce the cases. It includes the finding of the biomarkers of the cancer (Grizzle *et al.*, 2005), identification of gene mutations (Schulz *et al.*, 2003) as well as the role of Arachidonic Acid metabolism (Attiga *et al.*, 2000) through cyclooxygenase, lipoxygenase and P450 epoxygenase pathways (Nie *et al.*, 2002). In such cases, the treatment might be different from others.

Based on a case control study, it suggested that the use of Non-steroidal Anti-Inflammatory Drugs (NSAIDs) may help in reducing the risk of prostate cancer (Alan *et al.*, 1998) by inhibition of cyclooxygenase (COX) enzyme (Dempke *et al.*, 2001). Hence, prostaglandins mediators or inflammatory cells will be reduced. It is believed that the synthesis of eicosanoids through cyclooxygenase pathway played significant role in developing many cancers like colorectal cancer (Greenhough *et al.*, 2009). However, NSAIDs were advice not to be consumed by people with complications of gastrointestinal and cardiovascular as NSAIDs can cause topical injury on mucosal layer by which acidic properties of NSAIDs would dissociate into ionised form and trap hydrogen ions inducing mucosal injury (Sostres *et al.*, 2010). Moreover, it also can induce skin irritation as explained by Lee and Thomson (2006).

In recent years, the use of herbal medicines based has become more popular and being accepted not only by local market but also among western countries as it showed the fewer side effects and safer compared to the modern treatment (Mushtaq Ahmad *et al.*, 2009). Research studies has extensively focus on the antioxidant compounds of the plant as it was revealed to possess antibacterial, anti-inflammatory, anticancer, antiviral, anti-aging and others effects (Yizhong *et al.*, 2003). Antioxidants protect the cells from oxidative damage which later on if “not secured” will lead to many types of diseases such as cancer. Moreover, it is believe to enhance blood circulation of the body especially to the heart and brain by preventing injury to the blood vessel membranes (Olayinka *et al.*, 2012). Many studies has been conducted by researchers whereby it was proven that plants-containing polyphenols

compounds can be used in the prevention and treatment of cancer by the regulation of growth factor – receptor interactions and cell signalling cascade (Wahle *et al.*, 2010).

Generally, *Ficus deltoidea* (FD) belongs to the family Moraceae, genus Ficus and species of *deltoidea*. In Malaysia, it is known as Mas Cotek or sempit-sempit however in 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 but can also be found in Africa. According to Corner (1969), there are about 13 different varieties of *F. deltoidea* identified based on the morphological of leaves and figs. Nevertheless, three different varieties of *F. deltoidea* were selected in this study including var. *angustifolia*, var. *deltoidea* and var. *kunstleri*. This selection was based on the identification and confirmation made by botanist from ten plants which have been sent to Universiti Putra Malaysia. Furthermore, it has shown many pharmacological effects on human body for instances such as antimicrobial (Lee *et al.*, 2011), antinociceptive (Sulaiman *et al.*, 2008), antioxidant (Wahid *et al.*, 2010; Lee *et al.*, 2011; Norra, 2011) as well as anti-inflammatory (Zunoliza *et al.*, 2009; Zakaria *et al.*, 2011).

## 1.2 Problem Statement

Based on the studies conducted by Zunoliza *et al.* (2009), it was proven that *F. deltoidea* contains high levels of phytochemical called vitexin and isovitexin. Vitexin is a class of lignan compounds and it is part of flavonoid glycoside found in certain plants (Sahreem *et al.*, 2013). As previously described (Zhao *et al.*, 2009), vitexin induced apoptosis as well as suppress tumour growth. Although the numbers of experimental studies has reported the pharmacological effects of *F. deltoidea*, it is difficult to find a study based upon the relationship between antioxidant and anticancer activities in view of the prostate cancer cell line. Moreover, it is not known whether each fraction of *F. deltoidea* extract would be able to give similar positive effect to the prostate cancer and which varieties of *F. deltoidea* is have the

highest prostate cancer inhibition properties. The proposed project is expected to yield novel insight on the effect of *F. deltoidea* extract on cancer and likely lead to better understanding of anticancer properties of *F. deltoidea* and ultimately a product which could prevent cancer such as prostate cancer.

### **1.3 Objective of the Research**

The objectives of this study are:

- 1) To evaluate the potential properties of three different varieties of *F. deltoidea* on prostate cancer cell in view of antioxidant activities.
- 2) To determine the best fraction among three different varieties of *F. deltoidea* extracts for prostate cancer cell inhibition.
- 3) To evaluate anticancer activity of three different varieties of *F. deltoidea* and its fractions for prostate cancer cell.

### **1.4 Scopes of the Research**

In order to study the anti-cancer effects of three different varieties of *F. deltoidea* against prostate cancer cell line (DU145), these aqueous extracts of three different varieties of *F. deltoidea* were subject to several processes. It included the determination of antioxidant activity through total phenolic and flavonoid content methods and free radical scavenging activity analysis at various concentrations.

Next, the evaluation of anti-cancer effect of aqueous extract of three different varieties of *F. deltoidea* was evaluated at concentrations of 0.08 mg/mL – 2.4 mg/mL by which 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was conducted. Then, the best varieties was identified and proceed for fractionation using Solid Phase Extraction (SPE) method whereby different concentrations of ethanol-water as eluents were used ranging from 20% to 99.8%. The fractions of *F. deltoidea* var. *kunstleri* were determined its total phenolic and flavonoid content analysis along with cytotoxicity study, MTT assay at 100 µg/mL. The phytochemical profiling of the best fraction of *F. deltoidea* var. *kunstleri* that exhibiting the best anticancer activity on DU145 and antioxidant activity were evaluated on its phytochemical profiling using High Performance Liquid Chromatography (HPLC) and Fourier Transform Infrared Spectroscopy – Attenuated Total Reflectance (FTIR-ATR).

### **1.5 Research hypothesis**

1. The three different varieties of *F. deltoidea* possess different antioxidant properties in which it is believed to exert anti-prostate cancer.
2. If *F. deltoidea* aqueous extract is showed good anti-prostate cancer property, then the fractions of the extract will also produce a similar positive result by its structure function relationship mechanism.



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