PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF FRUITS OF *FICUS DELTOIDEA* VAR *ANGUSTIFOLIA* SP.

(Kandungan Fenolik dan Aktiviti Antioksidan bagi Buah *Ficus Deltoidea* var *Angustifolia* sp.)

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Abstract

The aim of the study was to evaluate the antioxidant activities from the fruits of Ficus deltoidea (var angustifolia sp.). This experiment used successive extraction method for the extraction process using three solvents of different polarity (hexane, chloroform and methanol). The antioxidant activity was determined using ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods while the radical scavenging activity was measured by 1,1-diphenyl-2-picrylydrazyl (DPPH) method. Folin-Ciocalteu reagent was used to estimate the total phenolic content. The hexane extract showed higher TPC value (259.2 mg/g GAE) followed by methanol extract (245.2 mg/g GAE) and chloroform extract (159.2 mg/g GAE). In the DPPH test, the extracts of methanol and chloroform showed more than 50% free radical scavenging activity at 250 µg/mL and 125 µg/mL concentration respectively. All extracts showed strong antioxidant activity in the TBA and FTC tests, with percent inhibition range from 90.70% to 97.78% respectively.

Keywords: Ficus deltoidea var angustifolia, antioxidant activity, FTC, TBA, DPPH

Abstrak

Tujuan kajian dijalankan adalah untuk menilai aktiviti antioksidan bagi buah *Ficus deltoidea* (var *angustifolia sp.*). Ekstrak secara berperingkat menggunakan tiga pelarut berbeza kepolaran (heksana, klorofom dan methanol) telah dijalankan. Aktiviti antioksidan ditentukan melalui ujian ferik tiosianat (FTC) dan asid tiobarbiturik (TBA), manakala 2,2-difenil-1-pikrilhidrazil (DPPH) digunakan untuk menguji penangkapan radikal. Kandungan fenolik total ditentukan menggunakan reagen Folin-Ciocalteu. Ekstrak heksana menunjukkan kandungan fenolik total yang tinggi (259.2 mg/g GAE) diikuti oleh metanol (245.2 mg/g GAE) dan klorofom(159.2 mg/g GAE). Bagi ujian DPPH, ekstrak metanol dan klorofom menunjukkan aktiviti penangkapan radikal bebas melebihi 50% pada kepekatan 250 µg/mL dan 125 µg/mL. Semua ekstrak menunjukkan aktiviti antioksidan yang tinggi dengan peratus perencatan dalam julat 90.70% ke 97.78%.

Kata kunci: Ficus deltoidea var angustifolia, aktiviti antioksidan, FTC, TBA, DPPH

Introduction

Free radicals are highly reactive and unstable compounds produced in the body during normal metabolic functions or introduced from the external environment such as pollution and cigarette smoke. Human bodies are protected from oxidative damage of free radicals through some complex defense systems which are called antioxidants. Antioxidant works to maintain the oxidant at optimum level and to reduce free radical, stopping it from forming before it can disrupt living cells in our body. However, excessive oxidants or free radicals can cause cell damage and lead to chronic diseases.

Studies show that diets high in antioxidants or antioxidant supplements reduce cancer death rates, cold and flu infections and protect against atherosclerosis, heart disease and cataracts. Antioxidant vitamin may improve immune system functions and may even delay some of the effects of aging [1]. Various antioxidants have been identified such as ascorbic acid, β-carotene and α-tocophenol [2]. Naturally-occurring antioxidants are Vitamin E, Vitamin C and β-carotene. More researchers have begun to formally study the health benefits of herbs and spices. In general, fresh herbs and spices are healthier and contain higher antioxidant levels compared to their processed counterparts [1].

*Ficus deltoidea (*var *angustifolia sp.)* or known as "Mas Cotek" [3] is one of the herbal plants that can be found in Malaysia. This plant is also found in Africa, Indonesia, and Southern Philippines. Each part of the plant is known to have medicinal properties [4] such as reducing level of sugar in blood, decreasing blood pressure, reducing cholesterol and lipids, migraine, contracting the vagina after delivery, delaying menopause and

reducing the risk of cancer. Furthermore, previous researcher has reported that the plant showed positive antinociceptive activities [5]. Meanwhile, another study has found that a female leaves of the plant is better than male leaves in term of antioxidant potential [6]. The main objective of this study was to compare phenolic content and free radical scavenging activity the fruits of *Ficus deltoidea* var *angustifolia* extracted successively from hexane, chloroform and methanol.

Experimental

Plant Material and Extraction

Fruits of *Ficus deltoidea* var *angustifolia* sp. were collected from nature forest near Lata Kinjang, Tapah, Perak. The variety was kindly confirmed by Mr Shamsul Khamis, a plant taxonomy specialist from Institute of Biological Sciences, University Putra Malaysia. Fruits of the plant were cut into small pieces and dried at room temperature. The sample was then ground to a fine powder. The material was extracted by successive soaking for three days using hexane, chloroform and methanol. The solvent extracts were filtered using muslin cloths and cottons. The solvents were removed using the rotary evaporator and were ready for further investigation.

Total Phenolic Content (Folin-Ciocalteu Method)

The total phenolic content was determined in all the three extracts following the Folin-Ciocalteu method [7]. The absorbance was measured at 760nm using UV/Vis spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrammes per gramme of dry material

DPPH Radical Scavenging Activity Assay

Free radical scavenging activity of the plant extracts was determined by the method of Yamaguchi *et al*. (1998) [8]. Briefly, 1.5 ml of DPPH solution (0.1 mm, in 95% ethanol) was incubated with varying concentration of the extract (0.75-5.0 mg). The reaction mixture was shaken well and incubated for 20 min at room temperature and the absorbance of resulting solution was read at 517 nm against a blank. Quercetin and BHT were used as standard references. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$Scavenging\ effect\ (\%) = [1- (A_{sample} / A_{control})] \times 100$$

Ferric Thiocyanate Method

The FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. The FTC assay was carried out as described by Kikuzaki and Nakatani (1993) [9]. A mixture of 4 mg of extracts (final concentration 0.02% w/v) in 4 ml of 99.5% ethanol, 4.1 ml of 2.51% linoleic acid in 99.5% ethanol, 8.0 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water, contained in a screw-cap vial (Φ 38 x 75 mm) was placed in an oven at 40^{o} C and incubated in the dark. To measure the extent of antioxidant activity, 0.1 ml of the reaction mixture was transferred into a test tube (Φ13 x 150 mm). Then, 9.7 ml of 75% (v/v) aqueous ethanol was added to it, followed by 0.1 ml of 30% aqueous ammonium thiocyanate and 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid. Three minutes after the addition of ferrous chloride to the reaction mixture, the absorbance was measured at 500 nm. The measurement was taken every 24 hours until the absorbance of the control reached its maximum value. Vitamin E was used as a positive control.

Thiobarbituric Acid Method

The test was conducted according to the method of Kikuzaki and Nakatani (1993) [9]. The same samples prepared for FTC method were used. To 2.0 ml of the sample solution, 1.0 ml of 20% aqueous trichloroacetic acid (TCA) and 2.0 ml of aqueous thiobarbituric acid (TBA) solution were added. The final sample concentration was 0.02% w/v. The mixture was placed in a boiling water bath for 10 minutes. After cooling, it was then centrifuged at 3000 rpm for 20 minutes. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on the absorbance of the final day of the FTC assay. Both methods (FTC and TBA) described antioxidant activity by percent inhibition:

$$\%\ \ inhibition\ = \frac{Absorbance\ \ of\ \ control\ - Absorbance\ \ of\ \ sample}{Absorbance\ \ of\ \ control} \times 100$$

Results and Discussion

The percent yield and total phenolic compounds in hexane, chloroform and methanol extracts of fruits of *F. deltoidea* var *angustifolia* are shown in Table 1. The weight of the crude extracts obtained was in the range of 28.4 g to 54.7 g corresponding to a percent extraction yield of 4.6% to 9.0%. The percent yield increase with the increase of the polarity of solvent. Methanol extract indicates the highest percent yield (9.0%) compared to chloroform (6.5%) and hexane extracts (6%).

Table 1: Extraction and total phenolic content of fruit extracts.

Solvent	Crude extract (g)	Extraction (%)	TPC (mg/g) GAE
Hexane	28.4	4.6	259.2
Chloroform	39.9	6.5	159.2
Methanol	54.7	9.0	245.2

*weight of dried fruits of *Ficus deltoidea* (var *angustifolia sp*.) is 610.4 g

In the present study, the total phenolic content of fruits of *Ficus deltoidea* var *angustifolia sp*. was determined by extrapolation from the calibration curve prepared from gallic acid concentrations and expressed in milligrams of gallic acid (GAEs). Figure 1 displays the total phenolic content of three different extracts of fruits of *Ficus deltoidea* var *angustifolia.* It was found that the hexane extract showed the highest phenolic concentration of 259.2 mg/g of GAEs followed by methanol extract which was 245.2 mg/g of GAEs. Low phenolic concentration was found for chloroform extract (159.2 mg/g of GAEs).

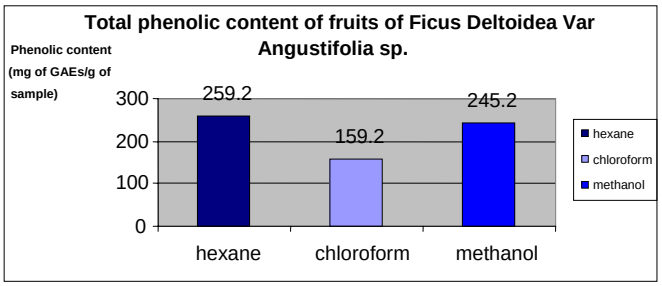

Figure 1: Total phenolic compounds in hexane, chloroform and methanol extracts of fruits of *Ficus deltoidea* (var *angustifolia sp*.)

In the DPPH radical scavenging assay, all the results obtained from the extracts of fruits of *Ficus deltoidea* (var *angustifolia sp*.) were compared with quercetin and BHT as standard references. As illustrated in Figure 2, all extracts show a gradual increase in activity with increase of concentration. The extract that can lower the initial absorbance of DPPH solution by 50% has been chosen as the endpoint for measuring the antioxidant activity. In this study, highest DPPH scavenging activity was shown by methanol extract at 250 µg/mL followed by chloroform at 125 µg/mL concentration.

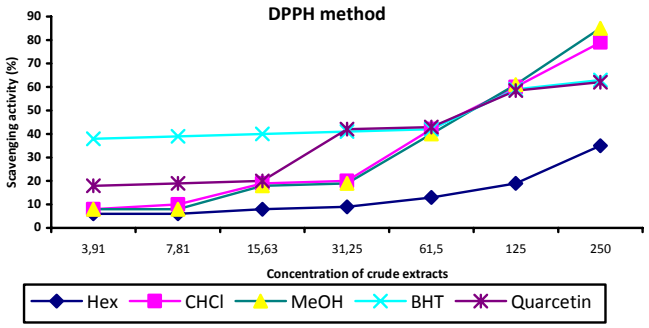

Figure 2: Scavenging activities of extracts with different concentration.

FTC method measured the amount of peroxide produced at the initial stage of lipid oxidation, whereas the TBA method measured at a later stage of lipid oxidation when peroxide decomposes to form carbonyl compounds. In the FTC method, all extracts exhibited strong antioxidant potential with percent inhibition in the range of 93% – 99% as compared with Vitamin E. Figure 3 illustrated the absorbance versus incubation time showing the activities of all crude extracts compared to standards for seven (7) consecutive days. Low absorbance correlate to high antioxidant activity. The highest percent inhibition is shown by hexane extract (97.78%) followed by methanol extract (96.69%) and chloroform extract (93.53%).

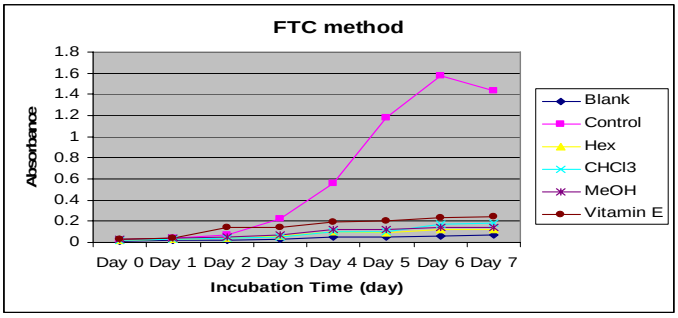

Figure 3: Antioxidant activities as measured by the FTC method.

Using the thiobarbituric acid (TBA) method, all extracts also showed strong antioxidant activities within the range of 91-96 % (Figure 4). The pattern of activity was similar for both FTC and TBA methods.

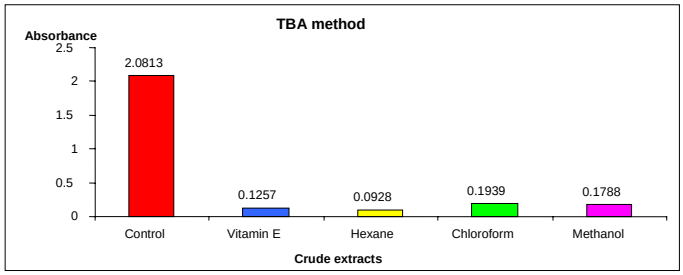

Figure 4: Antioxidant activities as measured by the TBA method.

Figure 5, show the total antioxidant activity of the FTC method as compared to the TBA method in which the activity of antioxidant for FTC is higher than the TBA method. This indicates that the amount of peroxide in the initial stage of lipid peroxidation is greater than the amount of peroxide in the secondary stage.

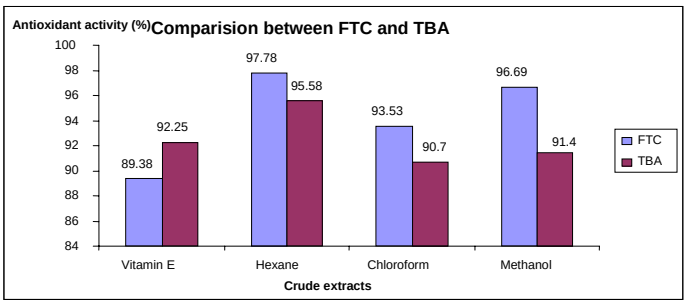

Figure 5: A comparisons between antioxidant activity of the three extracts using FTC and TBA methods.

In conclusion, the hexane extract from the fruits of *Ficus deltoidea* (var *angustifolia sp*.) displayed highest antioxidant activity followed by methanol extract and chloroform extract. This study demonstrates that the fruits of *Ficus deltoidea* var *angustifolia sp*. is a good source of antioxidant.

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