

Effect of Ascorbic Acid, Alpha-Tocopherol, Beta-Carotene and Total Polyphenol on Antioxidant Activity in Selected Malaysian Edible Plants

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ABSTRACT Malaysian edible plants have played a significant role in maintaining human health. These usually associated with the presence of antioxidants like ascorbic acid, α -tocopherol, β -carotene and polyphenols in these plants. The objective of this study was to screen selected different types of Malaysian edible plants; tapioca shoots (*Manihot utilissima*), kesom (*Polygonus minus*), selom (*Oenanthe javanica*), mint (*Mentha arvensis*), cekur manis (*Sauropus andrygonus*) and ulam raja (*Cosmos caudatus*) for antioxidant compounds and relate it to its antioxidant activity. β -carotene and α -tocopherol were determined using hexane-ethyl-acetate extraction method while, ascorbic acid, total polyphenol and antioxidant activity were determined using titration, spectrophotometer and β -carotene bleaching method, respectively. Ulam raja, kesom, tapioca shoot and cekur manis contained significantly ($p < 0.05$) the highest concentration of β -carotene (922.26mg/100g), total polyphenol (109.96 mg/100g), ascorbic acids (192.89 mg/100g) and α -tocopherol (19.23 mg/100g), respectively. However, kesom contained significantly the highest antioxidant activity (42.24%). Therefore, the presence of polyphenols in edible plants significantly increases its antioxidant activity properties when compared to the presence of other antioxidant compounds; β -carotene, ascorbic acid and α -tocopherol.

Keywords: edible plants, β -carotene, ascorbic acid, α -tocopherol, polyphenol, antioxidant activity.

ABSTRAK Tumbuhan yang boleh dimakan di Malaysia telah memainkan peranan yang penting didalam memperbaiki pemakanan manusia. Peranan ini dimainkan oleh wujudnya antioksidasi seperti asid askorbik, α -tocopherol, β -carotene dan polyphenol di dalam tumbuhan tersebut. Kajian ini dijalankan untuk menentukan askorbik asid, α -tocopherol dan kaitannya kepada aktiviti antioksidasi dalam tumbuhan terpilih yang boleh dimakan. β -carotene dan α -tocopherol dalam semua sample telah ditentukan menggunakan pengekstrakkan dengan Hexane-ethyl-acetate. Kepekatan asid askorbik, jumlah polyphenol dan aktiviti antioksidasi ditentukan menggunakan kaedah titrat, spektrofotometer dan pelunturan β -carotene. Ulam raja, kesom, pucuk ubi dan cekur manis masing-masing mengandungi kepekatan tertinggi ($p \leq 0.05$) untuk β -carotene (922.26mg/100g), jumlah polyphenol (109.96 mg/100g), asid askorbik (192.89 mg/100g) dan α -tocopherol (19.23 mg/100g). Kesom mempunyai aktiviti antioksidasi yang tertinggi ($p \leq 0.05$). Jadi, wujudnya jumlah polyphenol di dalam tumbuhan memberi kesan yang tinggi terhadap aktiviti antioksidasi jika dibandingkan dengan kompaun antioksidasi yang lain; β -carotene, asid askorbik dan α -tocopherol.

Kekunci kata: tumbuhan yang boleh dimakan, β -carotene, asid askorbik dan α -tocopherol, polyphenol dan aktiviti antioksidasi.

INTRODUCTION

The health benefits of consuming fruits and vegetables have been known for centuries and more recent epidemiological studies clearly

demonstrated that plant based food protect against several chronic diseases. The protective action of vegetable has been attributed to the presence of antioxidant compounds especially ascorbic acid, α -tocopherol, β -carotene and

polyphenols. Antioxidants are compounds that inhibit or delay the onset or slow the rate of oxidation. Each antioxidant compounds has their own role for the protection against free radical damage.

"Tocopherol" is a term that is used to identify a family of eight compounds consisting of α , β , γ , δ - tocopherol and the corresponding tocotrienols. α -tocopherol is a form of vitamin E most present in nature [1]. As an antioxidant, α -tocopherol is important to defend against cell membranes damage that are vital for healthy human. α -tocopherol can be found in many natural sources; green leafy vegetable, vegetable oils (sunflower, soybean and cottonseed), wheat germ, corn, nuts, seeds, olives, spinach and asparagus.

In recent years, great interest has been focused on antioxidant vitamin β -carotene because of its role in the prevention of heart disease and cancer [2]. β -carotene is the main source of vitamin A activity in most vegetables. Yellow and green vegetables or fruits are the main source of β - carotene, making these foods essential to human health. β - carotene is present in green leaves, where the presence of chlorophyll masks its colour.

Vitamin C or L-ascorbic acid in foods is easily oxidized to dehydro-L-ascorbic acid and both forms likely to be present in foods. Antioxidant vitamin C is widely distributed in nature and occurs in significant quantities in vegetable, fruit and in animal organs such as liver and kidneys.

Previous study had also indicated that antioxidant activities in fruits and vegetables are highly related to their total phenolic content [3]. Epidemiological studies have shown that consumption of food and beverages rich in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis by acting as antioxidant towards low-density lipoprotein [4].

Therefore, the objective of this study was to screen selected different types of Malaysian edible plants; tapioca shoots, kesom, selom, pudina, cekur manis and ulam raja for α - tocopherol, ascorbic acid, β -carotene and polyphenol and relate their presence to antioxidant activity.

MATERIALS AND METHODS

Sample Preparation

Edible plants selected for this study were "Ulam Raja" (*Cosmos caudatus*), "Selom" (*Oenanthe javanica*), "Pucuk Ubi" (*Topioca shoots*), "Cekur Manis" *Sauropus androgynus*), "Kesom" (*Polygonu minus*) and "Pudina" (*Mentha arvensis*). The fresh vegetables were washed and cut into small pieces. They were then dried in the vacuum oven at 60°C-70°C (20psi) for about 4-5 hours and ground to fine powder. The dried powder was kept at -18°C until needed for analysis.

α -Tocopherol and β -Carotene Content

The concentration of α - Tocopherol and β -carotene in selected edible plants were determined by using hexane-ethyl-acetate extraction method as described by [5].

Ascorbic Acid Content

Ascorbic acid analysis was carried out using 2, DCIP titration method [6].

Total Phenolic Content

Total polyphenol content was determined by using the method of [7]. 2g of sample were dissolved in 100ml 0.1N hydrochloric acid. The solution was stirred for 3 minutes, decanted for 5 minutes before it was filtered through Whatman no. 4. 50ml of steansy reagent, formaldehyde: hydrochloric acid: distilled water at the ratio of 7.5:5:5 (w/w/w) respectively was added to 50ml of the filtered solution. The mixture was boiled in reflux condenser for 1 hour and filtered again through predried filter paper (Whatman no. 4). The filter paper and the polyphenol compounds were dried in the oven at 50°C until constant weight was obtained.

Antioxidant Activity Assay: β - Carotene Bleaching Method.

Antioxidant activities of the plant extracts were determined according to the β -carotene bleaching method following a modification of the procedure described by [8]. The antioxidant activities of the samples were compared with synthetic antioxidants (α -Tocopherol and BHT; Sigma Chemical Co., St. Louis, MO). For a typical assay, 1ml of β -carotene solution, 0.2 mg/ml in chloroform, was added to 50 ml round-bottom flask containing 0.02ml of linoleic acid and 0.2ml

of Tween 20. Each mixture was then dosed with either 0.2ml of 80% MeOH as control, corresponding plant extracts or standards. After evaporation to dryness under vacuum at room temperature, 50 ml oxygenated distilled water was added and the mixture was shaken to form liposome solution. The samples were then subjected to thermal autoxidation at 50°C for 2 hour. The absorbance of the solution at 470 nm was monitored on a spectrophotometer by taking measurement at 10-minute intervals, and the rate of bleaching β -carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. About 50ppm of BHT and α -Tocopherol in 80% methanol were used as standard and 80% methanol was used as the control. All samples were assayed in triplicate.

The bleaching rate (R) of β -carotene was calculated according to Eq. (1)

$$R = \ln(a/b)/t \quad \text{Eq. (1)}$$

Where ln = natural log, a = absorbance at time 0, b = absorbance at time t and t = 20, 40, 60, 80 and 100

Antioxidant activity (AA) were calculated as percentage inhibition relative to control using Eq. (2).

$$AA = \frac{R_{\text{ontrol}} - R_{\text{sample}}}{R_{\text{ontrol}}} \times 100 \quad \text{Eq. (2)}$$

Statistical Analysis

All analysis were performed in triplicate. The data were recorded as means \pm standard deviation and analysed by SAS (Statistical Analysis System, 1996). Differences between means at 5% ($p < 0.05$) level were considered significant.

RESULTS AND DISCUSSIONS

Tocopherol is of great importance to human health because of their antioxidant properties. Edible plants contain high concentration of α -tocopherol [9]. From the results obtained, Cekur manis (*Sauropus androgynus*) contained significantly ($p \leq 0.05$) the highest α -tocopherol content (19.23mg/100g).

Ascorbic acid is widely distributed in nature and present in significant quantities in vegetable and fruits. Tapioca Shoots (*Manihot utilissima*) contain significantly the highest ($p \leq 0.05$) concentration of ascorbic acid, 192.89 mg/100g.

This results correlate well with the value in the Nutrient Composition of Malaysian Food (1998), which was 192 mg/100g.

Certain carotenoids posses vitamin A activity and of these β -carotene is the most widely spread and biologically most active. β -carotene is present in green leaves, where its colour is masked by that of chlorophyll. The concentration of β -carotene obtained from these samples was in the range of 12.22–922.26 mg/100 g edible potion of the plant. Ulam raja (*Cosmos caudatus*) contains significantly ($p \leq 0.05$) the highest amount of β -carotene.

It had been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds [8]. The antioxidant action of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donor, singlet oxygen quencher and metal chelators. In this study, total polyphenol expressed in term of percentage obtained from the samples were in the range of 3.31-9.72%. This results correlate well with Velioglu et. al., 1998 whereby they obtained in the range of 0.17-10.55 % from their study. Interestingly kesom have significantly ($p \leq 0.05$) the highest amount of total polyphenol, 9.72%, followed by selom, tapioca shoot, pudina, ulam raja and cekur manis.

Kesom also have significantly ($p \leq 0.05$) the highest antioxidant activity (42.24%). Ascorbic acid, α -tocopherol and β -carotene obtained for kesom in this study were very low. These results prove that the presence of α -tocopherol, ascorbic acid and β -carotene alone contribute little to antioxidant activity in these plants. However, a positive and a linear relationship between total polyphenol and antioxidant activity were observed in these plants ($r^2 = 0.6273$). [10] and [3] also obtained a linear relationship of polyphenol and antioxidant with r^2 value of 0.6578 and 0.963, respectively. Several studies also have indicated that the antioxidant activities of some fruits and vegetables are highly correlated to their total phenolic content [3, 10]. Numerous studies also have conclusively shown that antioxidant activity may be from compounds such as flavonoids, isoflavone, flavones, anthocyanin, catechin and isocatechin rather than from vitamin C, E and β -carotene.

CONCLUSION

The presence of ascorbic acid, α - tocopherol and β -carotene alone in these edible plants does not contribute much its to antioxidant activity. A combination of more than one antioxidant

compounds in the plant is responsible for its antioxidant activity. However, a positive and a linear relationship between total polyphenol and antioxidant activity were observed in these plants, $r^2=0.6273$.

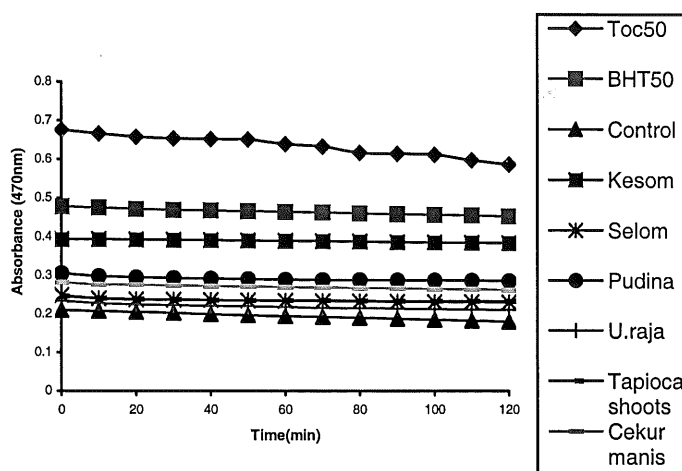


Figure 1. Antioxidant Activity in Selected Edible Plants Assayed by β -carotene Bleaching Method compared with synthetic antioxidants (BHT and Tocopherol) and control (80% methanol).

Table 1. The concentration of ascorbic acid, α - Tocopherol, β -carotene and total polyphenol in selected Malaysian edible plants.

Edible plants	Ascorbic acid (mg/100g)	α - Tocopherol (mg/100g)	β -carotene (mg/100g)	Total Polyphenol (g/100g)
Tapioca shoots (<i>Manihot utilisina</i>)	192.89 \pm 6.01 ^a	0.65 \pm 0.02 ^d	152.08 \pm 4.59 ^e	6.34 \pm 0.25 ^e
Cekur manis (<i>Sauropus androgynus</i>)	102.00 \pm 0.00 ^b	19.23 \pm 0.42 ^a	123.63 \pm 1.45 ^d	0.57 \pm 0.20 ^f
Selom (<i>Oenanthe javanica</i>)	68.33 \pm 10.41 ^c	10.16 \pm 1.22 ^b	394.72 \pm 10.08 ^b	7.66 \pm 0.26 ^b
Kesom (<i>Polygynus minus</i>)	45.87 \pm 9.10 ^d	0.65 \pm 0.13 ^d	45.00 \pm 1.55 ^e	9.72 \pm 0.47 ^a
Pudina (<i>Mentha arrensis</i>)	31.67 \pm 2.31 ^e	0.17 \pm 0.00 ^d	12.00 \pm 0.7 ^f	5.25 \pm 0.11 ^d
Ulam raja (<i>Cosmos caudatus</i>)	15.60 \pm 1.74 ^f	7.12 \pm 0.58 ^c	922.26 \pm 0.67 ^a	3.31 \pm 0.25 ^e

Mean values in the same column with different alphabets are significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test.

Table 2. Antioxidant Activity in Selected Edible Plants

Samples	Antioxidant Activity (%)
Tocopherol 50ppm	71.79 ± 0.00 ^a
BHT 50ppm	53.48 ± 0.00 ^b
Kesom (<i>Polygynus minus</i>)	42.24 ± 0.21 ^c
Pudina (<i>Mentha arrensisa</i>)	25.64 ± 0.00 ^d
Tapioca shoots (<i>Manihot utilisina</i>)	20.51 ± 0.00 ^e
Selom (<i>Oenanthe javanica</i>)	12.82 ± 0.00 ^f
Cekur manis (<i>Sauropus androgynus</i>)	12.70 ± 0.21 ^f
Ulam raja (<i>Cosmos caudatus</i>)	8.06 ± 0.00 ^g
Control	0.00 ± 0.00 ^h

Mean values in the same column with different alphabets are significantly different (p ≤0.05) according Duncan's Multiple Range Test.

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