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Determination of Ascorbic Acid, Total Phenolic Content and Antioxidant Activities in Turmeric Leaves of Different Maturity Stages

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Abstract A preliminary study was conducted to evaluate the ascorbic acid, total phenolic and antioxidant activities containing in turmeric leaves at different maturity stage. The total phenolic content, determined according to the Folin-Ciocalteu method was higher (77.4mg/100g) in mature than less mature turmeric leaf (55.2mg/100g). Antioxidant of methanolic extract evaluated according to the β-carotene bleaching method expressed as % inhibition relative to control were 81.7% in mature leaf and 13.5% in less mature leaf on dry weight basis. The ascorbic acid content analyzed using Hung & Yen method (2002) varied from 5.09 to 5.60 mg/100g in less mature and mature leaf. Results indicated a correlation between total phenolic content and antioxidant activities and higher amount of ascorbic acid content, total phenolic and antioxidant activities in mature than less mature turmeric leaf.

INTRODUCTION

Turmeric, also known as kunyit (*Curcuma domestica*) belongs to the Zingiberaceae family. It is widely distributed in tropical and subtropical regions especially in India, Thailand, China, Indonesia, Jamaica, Haiti and other tropical countries. Among the species cultivated, curcuma domestica is the most important as the source of turmeric since it has been widely used as condiments in foods. The part used is the rhizomes and the leaves. The leaf has a unique taste and smell and because of these important properties, it becomes part of the ingredients in Malay cooking [1]. It was found that turmeric also contains antioxidant compound, anti-fungal and anti-bacterial properties [2].

Antioxidants have traditionally been incorporated in foods because it has the ability to protect foods from rancidity and may preserve its color and flavor during storage [3]. Several researchers have investigated the antioxidant activity of flavonoids in different plants [4, 5, 6]. Earlier studies showed that total antioxidant activities were high in vegetables and fruits [7]. With increasing knowledge and awareness of the role of antioxidant in disease prevention, the search for naturally occurring antioxidant in food has created new research interest. This study is aimed at assessing the ascorbic acid, total phenolic and antioxidant activities in turmeric leaves since little information is available on the antioxidant content in this leaf.

EXPERIMENTAL

Plant Material and Sample Preparation: Turmeric leaves were purchased at local market (Section 16, Shah Alam). It was trimmed, washed and drained. The leaves were sorted according to its length and diameter: the length and diameter of the less mature leaves were 8.6cm and 11.5cm, and the mature leaves were 33.0cm and 48.3 cm respectively. Then the sample was dried in vacuum oven at 67±2° C (15 - 17Hg) for 4 hours. Finally it was ground into a fine powder and kept in freezer (-4°C) until further analysis.

Determination of Ascorbic Acid

Ascorbic acid was determined according to the method of [8] but with a slight modification. The sample powder (5g) was extracted with 100ml methanol, room temperature at 160rpm using an orbital shaker for 24 hours. The extract was filtered through Whatman No. 1 paper and the residue was further re-extracted twice with 100 ml methanol. The combined methanol extracts were then evaporated at 40°C using rotary evaporator. The dried methanolic extracts (20mg) were extracted with 10ml of 1% metaphosphoric acid for 45 minutes at room

temperature and filtered through Whatman No. 1 filterpaper. The filtrate (4ml) was then mixed with 9ml of 2,6-dichloroindophenol (DIP) and the absorbance was measured at 515nm within 15 seconds using UV-Visible Spectrophotometer. The content of ascorbic acid was calculated on the basis of the calibration curve of authentic L+ascorbic acid.

The standard solutions were prepared in various concentrations ranging from 10 to 60mg/L. Firstly, a stock solution (500ppm) of standard was prepared by diluting 50mg of ascorbic acid with methanol in 100ml volumetric flask. To prepare a 10ppm standard solution, 2ml of stock solution was marked up with methanol to 100ml. Then, 20ml of this solution was mixed with 10ml of 1% metaphosphoric acid (HPO_3). 4ml of the mixture was mixed with 9ml of 2,6-dichloroindophenol (DIP). The maximum wavelength was measured within the range of 400 to 650nm. The appropriate wavelength was fixed for further measurement of the absorbance of the sample.

Determination of Total Phenolics Content:
Total phenolics were determined using the Folin-Ciocalteau reagent [9]. The sample (200mg) was extracted with 2ml of 80% methanol containing 1% HCl at room temperature on orbital shaker set at 200rpm for 2 hours at room temperature. The mixture was then centrifuged at 1000rpm for 15 seconds and the supernatant was decanted into vials. The residue was re-extracted under identical condition. The extract (0.6ml) was mixed with 4.5ml of Folin-Ciocalteau reagent (previously diluted to 10-fold with distilled water) and allowed to stand at 22°C for 5 minutes. About 4.5ml of sodium bicarbonate solution (60g/L) was added to the mixture and the absorbance was measured at 750nm after 90 minutes.

The stock solution of 500ppm standard gallic acid was prepared. The various concentrations of standards (10 to 60mg/L) were prepared from the stock solution. Then, 0.6ml of the solution was mixed with 4.5ml of Folin-Ciocalteau reagent (previously diluted with distilled water). After that, 4.5ml of sodium bicarbonate solution (60g/L) was added. The maximum wavelength was measured within the range of 600 to 900nm. The fixed wavelength was used for further measurement of the absorbance in the samples.

Determination of Antioxidant Activities
Antioxidant activity of plant extracts and standards (α-tocopherol and BHA) were determined according to the β-carotene bleaching method [9]. For a typical assay, 1ml of 0.2mg/ml β-carotene solution in chloroform was added to a round-bottom flask containing 0.02ml of linoleic acid and 0.2ml of Tween 20. Each mixture was then added with 0.2ml of 80% methanol (control) or corresponding plant extract or standard. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50ml) was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autoxidation at 50°C for 2 hours.

The absorbance of the solution at 470nm was monitored on a spectrophotometer by taking measurements at 10 minutes interval. The concentration of BHA (50mg/L and 200mg/L) and α-tocopherol (50mg/L) in 80% methanol were used as standard and 80% methanol was used as the control. Antioxidant activity was calculated as % inhibition relative to control using the following equation:

$$AA\% = \frac{R_{control} - R_{sample}}{R_{control}} \times 100$$

Where $R_{control}$ and R_{sample} were the bleaching rates of β-carotene in reactant mix without antioxidant and with plant extract, respectively.

RESULTS AND DISCUSSION

Ascorbic Acid Content: The ascorbic acid content is slightly higher in mature leaves 5.60mg/L than in less mature leaves 5.09mg/L (Table 1).

Table 1: Means Value of ± SD of Ascorbic Acid, Polyphenolic Content and Antioxidant Activities (% inhibition) in Turmeric Leaves of Different Maturity

Sample	Ascorbic Acid (mg/100g)	Total Phenolic (mg/100g)	Antioxidant Activity (%)
Turmeric Leaves A	5.1^{b}	55.2^{b}	13.9^{b}
Turmeric Leaves B	5.60^{a}	79.4^{a}	81.7^{a}

Within the same column, averages with the same following letter are not significantly different ($p<0.05$).
TLA: less mature leaves
TLB: mature leaves

Total Phenolic Content: The total phenolics content in both mature and less mature leaves were 55.217mg/100g and 77.359mg/100g respectively, based on dry weight basis (Table 1). The phenolic content in mature leaves is significantly higher ($p<0.05$) than less mature leaves. Comparatively, turmeric roots contain about 175.5 ± 7.2mg/100g of dry weight basis phenolic content [10], which is higher than in the leaves. The higher content of total phenolic in turmeric roots as compared to the leaves is attributed to Curcumin (diferuloymethane), a phenolic compound present in *Curcuma domestica.* It is a yellow-coloured polyphenolic phytochemical which has been in use for a very long time for the treatment of swellings and wounds [11].

Antioxidant Activities: The absorbance of methanolic extracts of turmeric leaf, BHT (50ppm), BHT(200ppm), tocopherol (50ppm) and control (methanol 80%) were plotted against time as shown in Figure 1. Results in Figure 1 shows a similar pattern of absorbance decreasing over time for Tocopherol (50ppm) and Control. It shows that the antioxidant activity of tocopherol (50ppm) decreased rapidly during 30 minutes interval. However, oxidation was quite constant after 30 minutes to 120 minutes. On the other hand, less mature (TLA) and mature leaves (TLB) posses very high antioxidant activity where the bleaching rate of β-carotene almost constant from time 0 to 120 minutes intervals. This results indicated that the antioxidant compound in these turmeric leaves can act as an effective antioxidant in inhibiting degradation of β-carotene like BHT(50 ppm) and BHT (200ppm) (Figure 1).

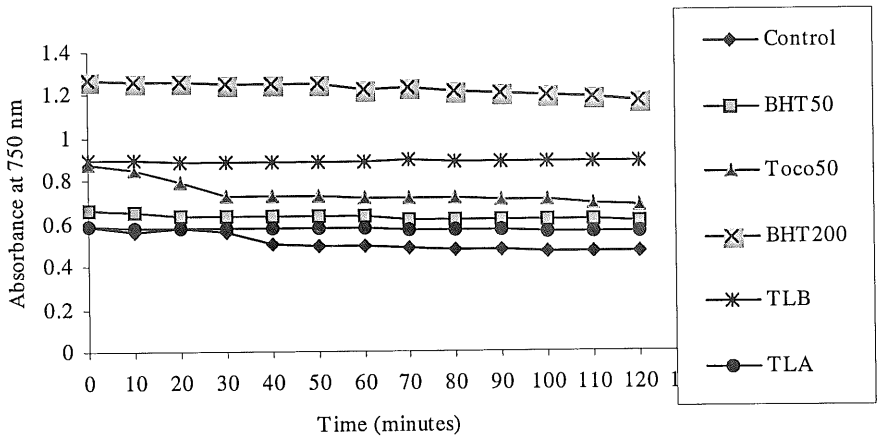

Figure 1: Antioxidant Activity of Turmeric Leaves Assayed by the β-carotene Bleaching Method.

The results also indicated that there is a correlation between antioxidant activities and total phenolic content in turmeric leaves. The antioxidant activities of turmeric leaves increase with the increase of total phenolic content, whereby, the higher the total phenolic content in turmeric leaves, the higher the antioxidant activities (Table 1). Recently, Velioglu et al. (1998) have demonstrated a linear relationship between antioxidant capacity and total phenolics in *Rubus* sp. and it was statistically significant between total phenolics and antioxidant activity ($R2=0.963$; $p<0.001$). These finding shows that mature leaves contains significantly ($p<0.05$) higher amount of ascorbic acid content, total phenolic content and antioxidant activity than less mature turmeric leaves. Furtherwork will be carried out to elucidate the identity of compounds responsible for the antioxidant activity in turmeric leaves.

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