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The *In Vitro* Antioxidant Activity of *Polyalthia Longifolia*

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Abstract. The current study was conducted to evaluate the antioxidant activity of methanolic (MeOH) extract from the leave *Polyalthia longifolia*. The antioxidant activity was evaluated by DPPH radical-scavenging activity, hydroxyl radical scavenging assay and reducing power assay. The commercial antioxidant butylated hydroxytoluene (BHT, Sigma), vitamin C was used for comparison or as a positive control. It was shown that *P. longifolia* extract tested was endowed with antioxidant activity. The antioxidant activity of the extract from leave was lower than the commercial antioxidant tested but still showed good antioxidant activity above 50%. The results of this study showed that *P. longifolia* extract as potentially novel sources of free radical scavenging compounds.

Keywords: *Polyalthia longifolia*, Antioxidant activity, Free radicals

1. Introduction

There is a strong need for effectual antioxidants from natural resources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases which can have serious effects on the cardiovascular system, either through lipid peroxidation or vasoconstriction Therefore, in this study, the antioxidant properties of the methanol extracts of leaves of *Polyalthia longifolia* was evaluated. *P. longifolia* var. angustifolia Thw. (Annonaceae), is a small medium-sized tree with linear-lanceolate leaves, 1 to 1.5 cm broad, occurring in Sri Lanka and now grown in tropical parts of India on road side and garden for their beautiful appearance [1]. *P. longifolia* is one of the most important indigenous medicinal plants and found throughout Malaysia and widely use in traditional medicine as febrifuge and tonic [2]. The diterpenes, alkaloids, steroid and miscellaneous lactones were isolated from its bark [3].The stem bark extracts and isolated compounds were studied for various biological activities like antibacterial, cytotoxicity and antifungal activity [3, 4].

2. Materials and Methods

2.1. Plant Collection and Extraction

Sample was collected from Penang, Malaysia, on January of 2012. Plant was identified by a botanist of School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. Leaves of selected plants were dried (room temperature) and powdered with a mortar.

2.2. Preparation of Plant Extracts

Some 100g of dried and powdered plant material was extracted at room temperature with 500 mL of methanol under constant shaking for 24 h. After filtration, the methanolic (MeOH) solutions were evaporated to dryness in a rotary evaporator for the antioxidant assays.

2.3. DPPH Radical-Scavenging Activity

P. longifolia extracts (50.0 µl) was mixed with 5.0 ml of methanolic solution containing DPPH radicals (0.004% w/v). The mixture was shaken vigorously and left to stand for 30 min in the dark (until stable

absorbance values were obtained). The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract is added at a particular level and A_{DPPH} is the absorbance of the DPPH solution [5]. BHT was used as standard.

2.4. Reducing Power Assay

The reducing power of extracts was determined according to the method of Yen and Chen [6] with a slight modification. Different concentration of extract (0.5 ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 ^oC for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to terminate the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), the reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm. All tests were performed six times. A higher absorbance of the reaction mixture indicated greater reducing power. BHT was used as a positive control.

2.5. Hydroxyl Radical Scavenging Assay

The capacity to scavenge hydroxyl radicals was measured according to a modification method of Klein et al. [7]. The reaction mixture contained 1 ml of iron-EDTA solution, 0.5 ml of EDTA (0.018%), 1 ml of DMSO (0.85% v/v in 0.1M phosphate buffer, pH 7.4) and 0.5 ml of ascorbic acid (0.22%). The tubes were capped tightly and heated in a water bath at 80-90 °C for 15 min. the reaction was terminated by adding 1 ml of ice-cold TCA (17.5%). Three ml of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone and adjust the volume up to 1L) were added to each tube and left at room temperature for 15 min for color development. The intensity of yellow colour was measured spectrophotometrically at 412 nm against blank sample. Ascorbic acid was used as a positive control. All tests were performed in triplicate. The hydroxyl radicals scavenging activity was calculated by following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the extract or positive control.

3. Results and Discussion

DPPH$^{\bullet}$ is a stable nitrogen-centred free radical the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [8].The first method used in this study was the free radical scavenging activity of DPPH. Values obtained for this DPPH assay are shown in Figure 1 with positive control BHT. As shown in Figure 1 the plant extract shows the highest DPPH radical scavenging effect with 82.3% scavenging activity (value of 75.3%). The DPPH radical scavenging effects of the standards was higher than the extract tested (Figure 1). However, the plant extract still showed better activity although we used crude extract. This implies that although there are plants with good antioxidant abilities further concentration and/or purification is needed to achieve better antioxidant capacities.

Different research has showed that the electron donation capacity of plants extract is associated with antioxidant activity. In this assay, the ability of extracts to reduce iron (III) to iron (II) was determined and compared to that of ascorbic acid, which is known to be a strong reducing agent. Figure 2 shows the reducing power of the *P. longifolia* extract. On average, methanolic extracts of *P. longifolia* had reducing power lower than those of positive control vitamin C. The reducing properties are generally associated with the presence of reductones [9], such as ascorbic acid (a potent reducing agent), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [10]. Reductones are also reported to react directly with peroxides [11] and also with certain precursors of peroxides, thus preventing peroxide formation [12]. Our data on the reducing power of *P. longifolia* extract suggested that the

antioxidant activity of *P. longifolia* extract likely involves other mechanisms in addition to those of reductones.

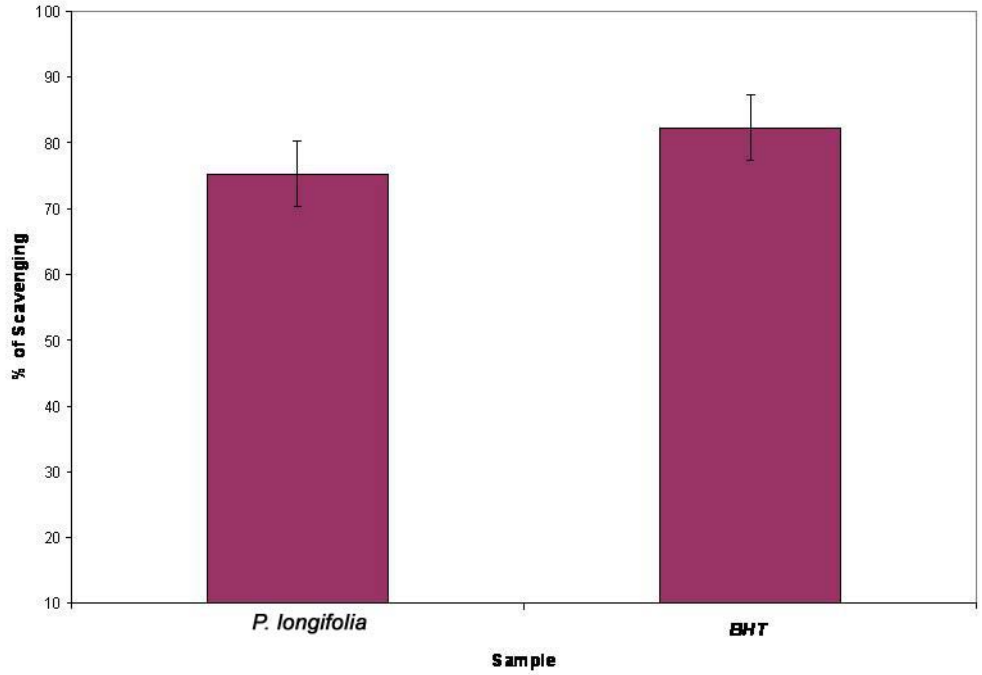

Fig. 1: Antioxidative effects (DPPH radical scavenging activity) of *P. longifolia* methanol extract and BHT

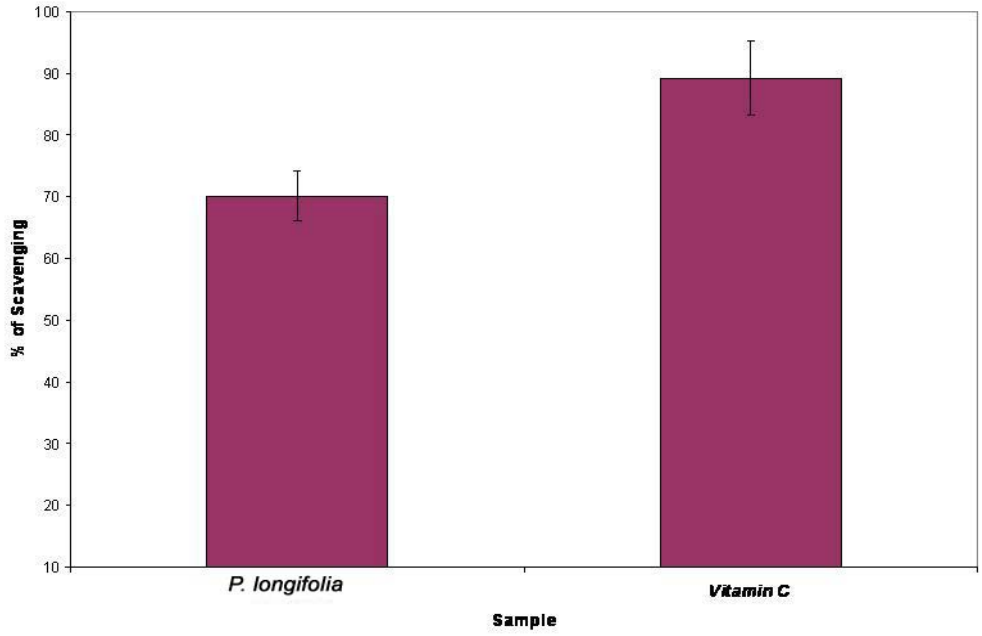

Fig. 2: Reducing power of *P. longifolia* extract

The evidence of the radical scavenging potential of the extract was further confirmed by investigating its ability to scavenge •OH radical. The efficacy of the *P. longifolia* extract to quench the hydroxyl radicals is shown in Figure 3. The scavenging ability of extracts on hydroxyl radical was lower compared with vitamin C. Hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. Although the *P. longifolia* extract showed a lower hydroxyl radical scavenging activity compared with vitamin C, but still can be considered as good starting material for the antioxidant compound.

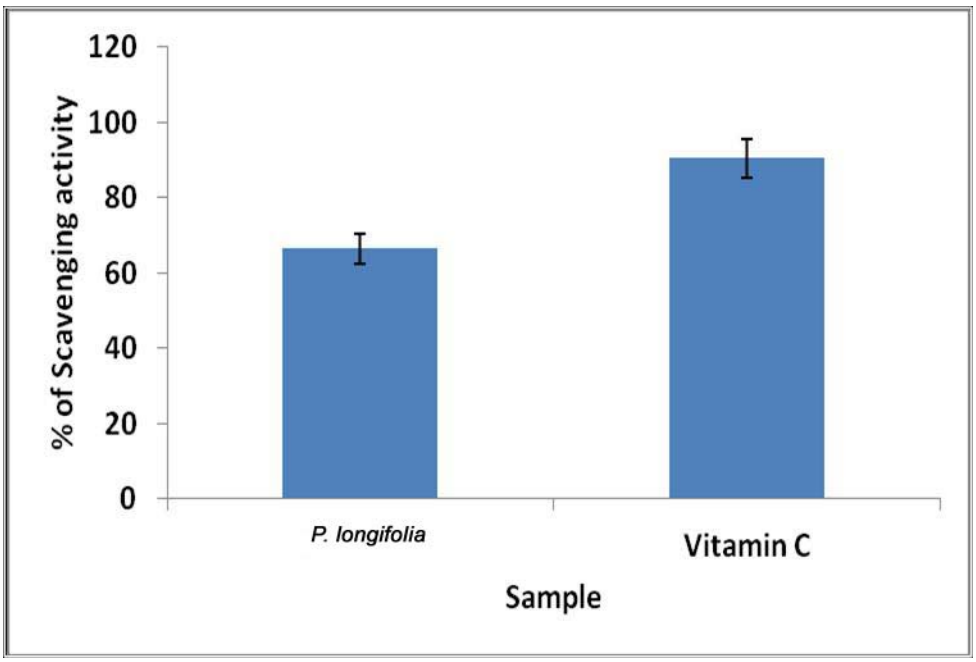

Fig. 3: Hydroxyl radical (OH•) scavenging ability of *P. longifolia* extract

4. Conclusion

The present study clearly established that *P. longifolia* extract has high promise as sources of natural antioxidants. There is lack of information available on the chemical composition of *P. longifolia,* which exhibit antioxidant activity. Further phytochemical work need to be done on these extracts including fractionation to isolate active constituent and subsequent pharmacological evaluation.

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6. References

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