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RESEARCH ARTCLE

EFFECTS OF LEAF MATURITY OF *Piper sarmentosum* (KADUK) ON ITS ANTIOXIDANT LEVEL

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ABSTRACT

Kaduk (*Piper sarmentosum*) is popular due to its culinary and medicinal properties and has been used traditionally in different parts of the world to cure many diseases and ailments. One of the studies which was conducted by Forest Research Institute of Malaysia (FRIM) shows that the extract from *P. sarmentosum* leaves contains antioxidant properties. However, a scientific study of its antioxidant level based on leaf maturity has not yet been conducted. Thus, this study aims to screen the antioxidant activity of *P. sarmentosum* based on the leaf maturity, from young, middle-age and matured leaves. The leaves were extracted by maceration using methanol and the antioxidant activity was determined by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. Results showed that the middle-age leaves contain the highest antioxidant activity followed by the young and matured leaves. Hence, if *P. sarmentosum*'s leaves are to be collected for its antioxidant properties, it is best to harvest the middle-age leaves to gain the optimized yield of antioxidant properties.

KEYWORDS

Piper sarmentosum, natural product, leaves maturity, antioxidant.

1. INTRODUCTION

Piper sarmentosum or known as kaduk in Malaysia is a climbing herb with long runners comes from the family of Piperaceae. The mature leaves are alternate and heart-shaped, while young leaves usually have waxy surfaces and are light green in color. The leaves are naturally aromatic and have a pungent smell [1]. *P. sarmentosum* has been traditionally used in culinary and alternative medicines in different parts of the world to cure many diseases and ailments. In Malaysia and Indonesia, the leaves and roots are used for treating headache, toothache, coughs, asthma, fungal dermatitis and pleurisy. In Thailand, the roots are used as carminative and stomachic, while the fruits and leaves are used as an expectorant [2].

Studies have been conducted to screen for the plant's nutritional and pharmaceutical properties. Research conducted by the Forest Research Institute of Malaysia (FRIM) shows that the extract from *P. sarmentosum* leaves possesses antioxidant properties [3]. Antioxidant compounds in 43 plants including *P. sarmentosum* were screen, and it was reported that the plant contain vitamin C, vitamin E, carotenes, xanthophylls, tannins and other phenolic compounds [4]. A different research showed that *P. sarmentosum* gave high superoxide scavenging activity, 88% when compared to superoxide dismutase (SOD) standard [5]. These studies confirmed that *P. sarmentosum* does have antioxidant activity in high levels. Meanwhile, the antioxidant activities of ethanol and aqueous extracts from the roots, stems, leaves and fruits of this plant, and showed that the extracts from the leaves part to contain the highest antioxidant levels [6].

The maturity of leaf may affect its antioxidant level, which gave different results of antioxidant level of berry crops' leaf according to the leaf maturity [7]. A research also proved that maturation of *Cosmos caudatus* leaf decreases its antioxidant capacity [8]. Hence, in this study, the leaf of *P. sarmentosum* is chosen to be further investigated for the effects of its leaf maturity on its antioxidant level. This study is important for both researches and agriculturalists to select the best leaf to be harvested whether for pharmaceutical production or vegetation purposes. Therefore, this research aims to study the antioxidant level of *P. sarmentosum* from leaf maturity to provide new information in determining the best stage of leaf to be harvested with the highest level of antioxidants properties.

2. MATERIAL AND METHODS

2.1 Collection of plant materials

Five kilograms of *P. sarmentosum* fresh leaves were collected at glasshouse and nursery complex in International Islamic University Malaysia, Kuantan. The samples were divided based on their maturity which were young, middle-age and old or matured leaves. This division was made based on their sizes and position (Figure 1), with young leaves being the smallest and at the tip position followed by middle age leaves at the middle position and old leaves at the bottom or lowest position.

Figure 1: From left; young leaf, middle-age leaf and old leaf

2.2 Preparation and extraction of *P. sarmentosum*

The leaves of *P. sarmentosum* were oven dried (45°C) for 3 days before being ground into powdered form with 30 g each. They were then

extracted using maceration method twice using absolute methanol as the solvent. For 30 g of samples, 300 mL of methanol was used for soaking and they were shaken for 5 hours before it was let to soak for 24 hours. The solutions were filtered using cheese cloth and the maceration method was then repeated. After maceration, the solutions were evaporated using rotary evaporator to remove the solvent and get the crude extract. Later, the crudes were weighed for 2 g for testing.

2.3 DPPH free radical scavenging assay

Briefly, stock solutions of plant extracts were prepared at 100 mg/mL concentration in absolute methanol. The samples were two-fold serially diluted to seven different concentrations (3.13 to 200 µg/mL) in microtiter well plate and were done in triplicate. Firstly, 100 µL of methanol was added to each well, followed by 100 µL of plant extracts to the first well before it was serially diluted. 100 µL of 0.01 mM of diphenylpicrylhydrazine radical (DPPH) was then added to each well. Next, the plates were shaken gently and incubated in the dark for 30 min before analysed using microplate reader. The absorbance was measured at 517 nm against ascorbic acid (vitamin C) as positive control and percentage of total radical scavenging activity was calculated using this formula of Equation 1:

DPPH radical scavenging activity (%) = $\frac{ODblank - ODsample}{ODblank} x\ 100\%$ Eq. 1

The values were plotted against the concentration of samples to get the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (EC_{50}), and these readings were compared between the three samples of leaves. The experimental results were analysed using one-way analysis of variance (ANOVA) and the differences between samples were determined by Post Hoc Test using Statistical Analysis System (SAS) program. *P*-value of <0.05 was regarded as significant.

3. RESULTS AND DISCUSSION

3.1 Antioxidant activity of *P. sarmentosum* leaves methanolic extract

It was observed that the methanolic extracts of the leaves had antioxidant activity based on the values of the percentage of inhibition for each leaf maturity (Table 1, 2 and 3). From the values, as concentration of samples increases, the percentage of oxidation inhibition also increases. This indicates that the concentration of samples is linearly proportional to the percentage of oxidation inhibition, and that the amount of antioxidant compounds increases with concentration.

Table 1: Percentage of oxidation inhibition for young leaves

Concentration	OD replicate 1	OD replicate 2	OD replicate 3	OD Mean	Percentage of inhibition (%)
200 µg/mL	0.1214	0.1334	0.1531	0.1340	80.91
100 µg/mL	0.3058	0.2829	0.3057	0.2981	57.54
50 µg/mL	0.4763	0.4277	0.4760	0.4600	34.48
25 µg/mL	0.5832	0.5757	0.5754	0.5781	17.66
12.5 µg/mL	0.6345	0.6388	0.6306	0.6346	9.61
6.25 µg/mL	0.6470	0.6380	0.5817	0.6222	11.38
3.13 µg/mL	0.6746	0.6777	0.6900	0.6808	3.03
Blank 0.0 µg/mL	0.6839	0.7075	0.7145	0.7021	-

Note: OD = optical density reading

Table 2: Percentage of oxidation inhibition of middle-age leaves

Concentration	OD replicate 1	OD replicate 2	OD replicate 3	OD Mean	Percentage of inhibition (%)
200 µg/mL	0.1017	0.1053	0.1021	0.1030	81.02
100 µg/mL	0.0994	0.0971	0.0932	0.0966	82.20
50 µg/mL	0.2745	0.2606	0.2665	0.2672	50.76
25 µg/mL	0.3999	0.3667	0.3893	0.3853	28.99
12.5 µg/mL	0.4656	0.4501	0.4600	0.4586	15.48
6.25 µg/mL	0.4885	0.4944	0.4983	0.4937	9.01
3.13 µg/mL	0.5174	0.5177	0.5201	0.5184	4.46
Blank 0.0 µg/mL	0.5419	0.5403	0.5456	0.5426	-

Note: OD = optical density reading

Table 3: Percentage of oxidation inhibition for old leaves

Concentration	OD replicate 1	OD replicate 2	OD replicate 3	OD Mean	Percentage of inhibition (%)
200 µg/mL	0.1511	0.1709	0.1640	0.1620	70.44
100 µg/mL	0.3199	0.3285	0.3315	0.3266	40.41
50 µg/mL	0.4280	0.4370	0.4307	0.4319	21.20
25 µg/mL	0.4862	0.4788	0.4856	0.4835	11.77
12.5 µg/mL	0.5214	0.5214	0.5072	0.5167	5.73
6.25 µg/mL	0.5322	0.5333	0.5244	0.5299	3.32
3.13 µg/mL	0.5412	0.5373	0.5167	0.5317	2.99
Blank 0.0 µg/mL	0.5484	0.5485	0.5474	0.5481	-

Note: OD = optical density reading

There are a few methods that can be used to determine the radical scavenging effects of antioxidants. However, the ideal one would be DPPH method as it is fast, easy, reliable and does not involved the need of special reaction and device [9]. DPPH is a stable free radical that can accept an electron or hydrogen radical transforming into a stable diamagnetic molecule with absorption band at 517 nm [10]. In the presence of antioxidant compound, DPPH solution will be converted to DPPH-H (diphenylhydrazine) molecules, resulting in discolouration of DPPH solution from purple to yellow due to the decreasing quantity of DPPH radicals [11].

Based on the obtained results, it can be concluded that the leaves of *P. sarmentosum* does have antioxidant properties regardless their level of maturity. This was supported by a research conducted that gave result of

377.41 mmol of antioxidant content in *P. sarmentosum* leaves [12]. However, the reading of antioxidant activity differs between young leaves, middle age leaves and old leaves, showing that age or maturity of leaves does affect the level of compounds responsible for antioxidant properties (Figure 2). This is in line with a study which revealed that leaf ontogeny (developmental stages) influences the biosynthesis and accumulation of secondary metabolites and thus their biological properties [13].

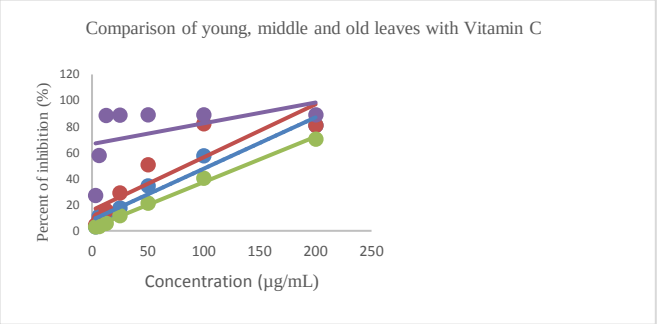

Figure 2: DPPH radical scavenging activity of the methanolic extracts of young leaves, middle-age leaves and matured leaves compared with vitamin C as positive control.

From the statistical analysis by using two-way ANOVA (analysis of variance), there is a significant difference between young leaves, middle age leaves and old leaves' level of antioxidant, with *p* value less than 0.05, and further post hoc test showed that each of them are significantly differ from each other. Based on the values of percentage of oxidation inhibition calculated for each sample, it can be concluded that middle age leaves has the highest antioxidant level, followed by young leaves and old leaves.

From the linear graph plotted, the values of EC_{50} (concentration required to obtain a 50% antioxidant effect) are calculated, a lower EC_{50} value indicates higher DPPH radical-scavenging activity [14]. The EC_{50} values for young leaves, middle age leaves and old leaves are 105.84 µg/mL, 42.32 µg/mL and 136.26 µg/mL respectively. From the EC_{50} values, it can be said that middle leaves has the highest DPPH radical-scavenging assay, followed by young leaves and old leaves.

A study to test antioxidant compounds in several plants in Thailand including *P. sarmentosum* and their correlation to antioxidant activity [4]. The compounds that have been screened in *P. sarmentosum* are vitamin C, vitamin E, total carotenes, total xanthophylls, tannins and total phenolics, and the results showed a high correlation of all these compounds with antioxidant activity. This means that younger leaves which are the middle-age leaves and young leaves contains more antioxidant compounds compared to mature or old leaves of *P. sarmentosum*.

A similar study was carried out on *Lantana camara*'s leaf position and its antioxidant compounds level and the results showed an increasing level of flavonoids and proanthocynidines from young to middle leaves before declining in matured leaves, and after being tested with DPPH free radical scavenging assay, the middle leaves gave highest antioxidant capacity compared to young and old leaves [13]. The result gave similar pattern of result with this present research which is the rising level of antioxidant activity from young leaves to middle leaves with a decrease in matured leaves. It can be concluded that the less antioxidant activity of old leaves may indicate its losing of secondary metabolites due to leaf senescence.

4. CONCLUSION

The leaf of P. sarmentosum does possess antioxidant properties. However, the antioxidant activity varies based on the leaf maturity or position. The level of antioxidant increases from young to middle age leaves but declines in old or matured leaves. Thus, if *P. sarmentosum*'s leaves are to be harvested for its antioxidant properties, it is best to select the middle age leaves to gain the optimized level of its antioxidant properties. Further research to be suggested is to screen the amount of antioxidant compounds in each leaf maturity in order to find the significant compounds that contribute to the variation of antioxidant level.

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