

ANTIOXIDANT PROPERTY ON VARIOUS LEAF MATURITY OF PIPER SARMENTOSUM

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ABSTRACT

Piper sarmentosum known as Kaduk in Malaysia is a climbing herb with long runners comes from the family of Piperaceae. *Piper sarmentosum* has been traditionally used in culinary and alternative medicines in different parts of the world to cure many diseases and ailments. Thus, this study aims to screen antioxidant activity of *P. sarmentosum* based on the leaf maturity, from young, middle-matured and matured leaf. First and foremost, the leaf will be extracted in 70% methanol and the antioxidant activity was determined by using Ferric Reducing Antioxidant Power (FRAP) assay. Result showed that the young leaves contain the highest antioxidant activity rather than middle-matured and matured leaves. Hence, if *P. sarmentosum*'s leaves are to be collected for its antioxidant properties, it is best to harvest the young leaves to gain the optimized yield of antioxidant properties.

KEYWORDS

Piper sarmentosum, kaduk, FRAP Assay



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INTRODUCTION

P. sarmentosum is an edible plant that can be eaten as a food ingredient after having cooked or bring to boil. In Malaysia and Indonesia, this cuisine is defined as Ulam, a kind of food that Asian usually have as a side dish with meal. *P. sarmentosum* has three forms of plant parts that include stems which are crawling, climbing and branching. This plant has creeping and rising stems and have several or same various shapes of the leaves. Leaves physiology for all plants forms such as size and pattern is quite distinctive (Chaveerach, Mokkaumul, Sudmoon, & Tanee, 2006). *P. sarmentosum* comprises many medical benefits based on studies performed by some researchers like antibacterial (Zaidan et al., 2005), antifungal (Rukachaisirikul et al., 2004), and antioxidant (Chanwitheesuk, Teerawutgulrag, & Rakariyatham, 2005).

Antioxidant significantly help to delay or prevent oxidation of oxidizable substrates been present at lower concentrations than the substrates (Halliwell, 2007). Plants also have been long a source of exogenous antioxidants and it is believed that two-thirds of the world's plant species have medicinal importance, and almost all of these excellent antioxidant potential (Krishnaiah, Sarbatly, & Nithyanandam, 2011). 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.

A study on different leaf maturity of *Cosmos caudatus* proved that the antioxidant level decreases as it leaves matured (Fatanah, Abdullah, Abdullah, Hashim, & Hamid, 2016). This indicate that maturity of leaf may affect its antioxidant level, which gave different results of antioxidant level of the leaf maturity. This study is important for both researchers 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 antioxidant properties.

RESEARCH METHOD

In this study, the antioxidant activity of *P. sarmentosum* was carried out by using methanol for extraction. To evaluate the antioxidant activity of *P. sarmentosum*, Ferric Reducing Antioxidant Power (FRAP) assay was done. For FRAP assay, we need to prepare FRAP reagent and FRAP solution. FRAP reagent were prepared from 300 mM acetate buffer, 10 mM TPTZ solution and 20 mM iron (II) chloride solution. To prepare, 300 mM acetate buffer, 8 ml of acetic acid was added with 1.6 g of sodium acetate and make up to 500 ml with pH 3.6. As for the TPTZ solution and iron (II) chloride solution, we need to use 1.57 g of TPTZ diluted in 40 mM HCl (1.7 ml of HCl dilutes with distilled water until 500 ml) and for 20 mM iron (II) chloride solution, we need 2.7 g of FeCl₃ diluted with distilled water until 500 ml. Then, the FRAP reagent was made in proportion of 10:1:1 (10 ml acetate buffer: 1 ml TPTZ : 1 ml FeCl₃.6H₂O) respectively. FRAP reagent was freshly prepared and warmed at 37°C for 5 minutes.

The standard solution which 1.0 mM FeSO₄.7H₂O was prepared by weight 0.4 mg of FeSO₄ and diluted it with distilled water according to the concentration desired (5 μM, 10 μM, 20 μM, 40 μM, 60 μM, 80 μM and 100 μM). Next, 3 ml of FRAP reagent was added to the 1 ml of each concentration of FeSO₄.7H₂O. Next for determination of antioxidant in each extract, 3 mL of FRAP reagent, 330 μL distilled water and 70 μL of the extracts were put into test tubes and inverted to ensure mixing. The extracts then left for 30 minutes in dark room before absorbance was measured at 593nm. The percentage reduction activity will be calculated using the following equation:

$$\% \text{ Reduction ability} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

The data were statistically calculated Microsoft Excel. The test determination was done in triplicate and report as mean values and standard deviation.

RESULT AND DISCUSSION

The FRAP assay results were used to determine and compare the antioxidant capacity of commercially available *P. Sarmentosum*. The maturity of *P. sarmentosum* leaves involved from young, middle-matured and matured leaves. Using the FRAP assay it was determined that which leaf maturity are by far the most powerful reducing agents. By observing the reduction of the pale blue Fe³⁺-TPTZ complex in the FRAP reagent to indigo coloured of Fe²⁺-TPTZ complex which absorbs strongly at 593 nm. The Fe²⁺-

TPTZ concentration was determined from absorbance value and the calibration of the absorbance against a solution known as Fe²⁺.

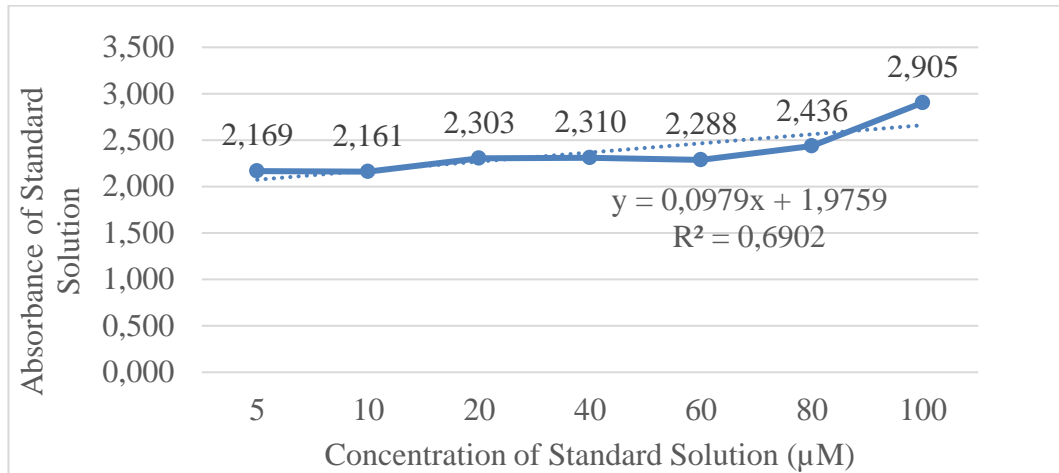


Figure 1 Standard curve of standard solution

Based on Figure 1, shows that the increase in values of absorbance of ferum sulphate, FeSO₄.7H₂O measured at 593 nm. The equation of the straight line was $y = 0.0979x + 1.9759$ and the graph shows that it is linearly proportional which represents that as the concentration of FeSO₄.7H₂O increases, the absorbance reading also increases.

Table 1 Percentage of reduction ability of FRAP reagent in extraction samples

Extract sample	Absorbance Reading 1	Absorbance Reading 2	Absorbance Reading 3	Mean Absorbance	Percentage Reduction Ability (%)
Blank	2.382	2.406	2.361	2.383	-
Matured Leaf	2.277	2.955	2.938	2.723	14.27
Middle Leaf	2.973	2.976	2.309	2.753	15.53
Young Leaf	2.985	2.306	2.982	2.758	15.74

Based on Table 1, the percentage of reduction ability of young leaf is 15.74% which shows higher than matured and middle-matured leaf of P. Sarmentosum. As for middle-matured, it shows higher than matured leaf with percentage of 15.53% rather than 14.27% for matured leaf.

The percentage reduction ability calculated in Table 4.2 using the following equation:

$$\% \text{ Reduction ability} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

Where:

Absorbance control = Mean of blank sample

Absorbance sample = Mean of extract sample

Matured Leaf:

$$\frac{2.383 - 2.723}{2.383} \times 100\% = -14.27$$

$$|-14.27| = 14.27\%$$

Middle-matured leaf:

$$\frac{2.383 - 2.753}{2.383} \times 100\% = -15.53\%$$

$$|-15.53| = 15.53\%$$

Young leaf:

$$\frac{2.383 - 2.758}{2.383} \times 100\% = -15.74\%$$

$$|-15.74| = 15.74\%$$

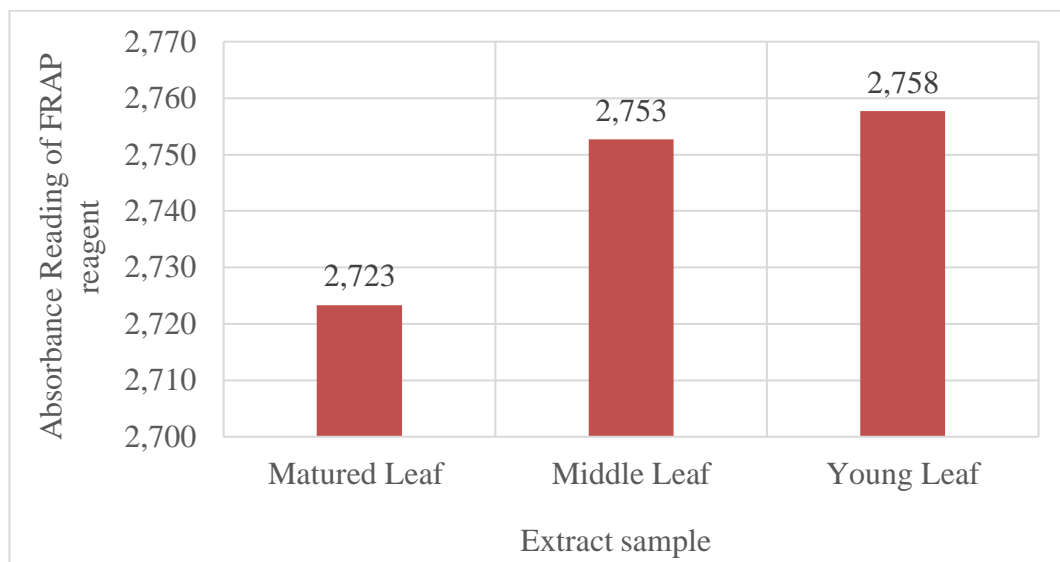


Figure 2 Antioxidant activity of each *P. sarmentosum* leaves

As for Figure 2 which is antioxidant activity of each maturity of *P. sarmentosum* with FRAP reagent shows that matured leaf has the lowest antioxidant level which is 2.729 while middle leaf and young leaf are 2.753 and 2.758.

Medicinal plants are crucial to the health of individuals and the communities. The medicinal values of these plant lie in some chemical substances that produce a definite physiological action on human body. Many herbal plants contain antioxidant compounds which protects cells against degenerative effect of Reactive Oxidative Stress (ROS) which is free radical such as singlet oxygen, superoxide, peroxy, radicals, hydroxyl radicals. The concept of oxidative stress is that when a balance between ROS production and antioxidant defence is lost, oxidative result which through a series of events deregulate the cellular function and leads to various diseases such as aging, arthritis, asthma, carcinogenesis, diabetes, rheumatism, and various neurodegenerative disease (Sanchez-Moreno, 2002).

Extraction is the main process by which bioactive compounds may be obtained from biomass materials. The objective of extraction process is to maximize the amount of target compounds and to obtain the highest biological activity of these extracts (Chang, Yang, & Wen, 2002). The extraction yield and biological activity of the resulting extract is not only affected by the extraction technique but also extraction solvent (Ajanal, Gundkalle, & Nayak, 2012). Due to variety of bioactive compounds contained in plant material and their differing solubility properties in different solvents, the optimal solvent of extraction depends on the particular plant material and compounds that are to be isolated (Ajanal et al., 2012).

This shows that antioxidant activity of the extract and extraction yields depends on the solvent that used during extraction process. In this research, methanol was chosen since it is a good polar solvent for extraction of most bioactive compound. It has higher polarity than chloroform, diethyl ether and other polar solvent.

In other hand, FRAP assay is to measures the ability of the antioxidants contained in the extracts to reduce Fe^{3+} -TPTZ complex to a blue coloured Fe^{2+} -TPTZ at low pH. Fe^{3+} is an oxidizing agent that accepts electrons and causes another reactant to be oxidized. Fe^{2+} which is produced from the reduction of Fe^{3+} in the FRAP assay, is a well-known pro-oxidant which is a reactive oxidative species (ROS) that can cause oxidative damage to lipids, proteins and nucleic acids, resulting in various pathologic events and/or diseases. It can react with H_2O_2 to produce OH, the most harmful free radical found in vivo. Therefore, the ability of the antioxidants in reducing Fe^{3+} may reflect their ability in reducing reactive species.

Based on the FRAP activity carried out, reducing ability of different leaf maturity of *P. sarmentosum* are presented in Table 4.2. Young leaf of *P. sarmentosum* extract ranked the first in exhibiting the most active ferric reducing ability compared to middle-matured and matured leaf (15.53% and 14.27%) accordingly, the order of ferric reducing ability obtained from this study is young leaf (15.74%) > middle-matured leaf (15.53%) > matured leaf (14.27%).

A study conducted by Ibrahim & Azman (2019), linear graph plotted showed The EC_{50} values for old leaves which is 136.26 $\mu\text{g}/\text{mL}$, lower than middle matured and young leaves which are 42.32 $\mu\text{g}/\text{mL}$ and 105.84 $\mu\text{g}/\text{mL}$ respectively. From the EC_{50} values, it shown that middle leaves have the highest antioxidant levels followed by young leaves and old leaves (Ibrahim & Azman, 2019) which this assay aims to calculate the 50% antioxidant effect which the a lower EC_{50} value indicates higher DPPH radical scavenging activity. Based on study conducted by Ibrahim & Azman (2019), the compounds that have been screened in *P. sarmentosum* are vitamin C, vitamin E, total carotenes, tannins and total phenolics, and the results showed a high correlation with high antioxidant activity. This showed that younger leaves which are categories as middle age leaves and young leaves contains high antioxidant compounds compared to matured or old leaves of *P. Sarmentosum*.

A similar study on *Lantana camara*'s leaf position and its antioxidant activity levels showed that middle leaves gave highest antioxidant capacity compared to young and old leaves (Bhakta-Guha & Ganjewala, 2009). The result gave similar pattern of result with this study 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 less antioxidant activity of old leaves may indicate its losing of secondary metabolites due to leaf senescence.

CONCLUSION

The FRAP reagent provides a useful tool for quick, inexpensive analysis of a given materials reducing capacity which is here why the FRAP assay is used to compare concentrations of reducing ability in various extracts form natural antioxidants. However, the main limitation of this method is that it cannot distinguish between reducing agents as the Fe²⁺ complex forming a blue solution after reduction from Fe³⁺ does not detect how the reduction occurred. This allows for simple comparison of reducing potential of various substances, but to identify the reducing agents and determine their relative concentrations, different analytical methods should be employed. Future research to be suggested is to screen the amount of phytochemical analysis in each leaf maturity to find the significant components that contribute to the variation of antioxidant level. Thus, future works need to be compared between phytochemical analysis and the antioxidant analysis of different leaf maturity of *P. Sarmentosum*.

As a conclusion, it can be concluded that leaves of *P. sarmentosum* does possess antioxidant properties regardless their level of maturity. However, the antioxidant activity varies based on the leaf maturity or position. The level of antioxidant increases from young to middle-matured leaves but declines on matured leaves. Thus, if *P. sarmentosum* leaves are to be harvested for its antioxidant properties, it is best to select the young leaves to gain optimized level of its antioxidant properties.

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