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RESEARCH ARTICLE

THE STUDY OF ANTIOXIDANT ACTIVITIES OF *PIPER SARMENTOSUM* AND *PIPER NIGRUM*

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ABSTRACT

Piper sarmentosum and *Piper nigrum* which belongs to family Piperaceae are well distributed in the tropical region including Malaysia. They are one of the medicinal plants which are well known for its health benefits to human. This study focused on determining the antioxidant activity of *P. sarmentosum* and *P. nigrum* leaves of ethanolic and aqueous extraction. For the extraction, different concentrations of ethanol and aqueous extracts were used. For the antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was conducted. The results showed that the scavenging activity of all extracts samples was in a concentration-dependent manner. High antioxidant activity of *P. sarmentosum* leaves was obtained by using ethanol extraction with 74% of inhibition, and IC50 value was 35.18 µg/mL. Meanwhile, P. nigrum leaves showed high antioxidant activity by using aqueous extraction with 64.68% of inhibition, and IC50 value was 79.89 µg/mL. It can be concluded that different extraction solvents used to give a different level of antioxidant activity of both *P. sarmentosum* and *P. nigrum.*

KEYWORDS

Piper sarmentosum, Piper nigrum, antioxidant, DPPH assay, extraction.

1. INTRODUCTION

Piper L. genus from Piperaceae family is economically important plants and beneficial for human health. There are 1200 species of *Piper* distributed in the pantropical and Neotropical region in the world with more than 400 species have been recorded in Malaysia region alone (Rahman et al., 2016). *Piper sarmentosum* and *Piper nigrum* are among the plants which play an essential role as a medicinal plant. *P. sarmentosum* is a wild-growing plant in the tropical forests and mostly in Asia region. The mature leaves are simple, alternate and heart-shaped. The young leaves consist of waxy surface and light green in colour. On the other hand, *P. nigrum* is a woody perennial climbing vine that grows in the tropical area which leaves and fruit-producing fragrance and essential oil. The plant leaves are simple, alternate, oval-shaped and leathery in texture. The upper leaves surface is dark green and whitish-green on the underside.

According to some study, *P. sarmentosum* and *P. nigrum* were claimed to possess anti-inflammatory, antioxidant, antimicrobial and anticancer properties (Rahman et al., 2016; Nahak and Sahu, 2011). Antioxidants or inhibitors of oxidation are the substances that can reduce the free radicals reaction and oxidation process. Free radicals are the molecules which have high reactivity, short half-life and damaging the macromolecule such as DNA, protein and lipids. The imbalance amount of free radicals leads to a various effect on the human body, such as cancer, ageing, and rheumatoid arthritis (Patel et al., 2013). Plants are the primary natural sources of antioxidants which beneficial to human health. Therefore, this research focuses on the identification of antioxidant level in leave samples of *P. sarmentosum* and *P. nigrum* extracts of different solvents. The objectives of the research were to extract crude *P. sarmentosum* and *P. nigrum* by using ethanolic and aqueous extraction, and secondly to analyze the antioxidant activities of *P. sarmentosum* and *P. nigrum* by using DPPH assay.

2. MATERIAL AND METHODS

2.1 Plant sample preparation

Fresh plant materials of *Piper sarmentosum* and *Piper nigrum* were collected from the garden of Taman Pertanian Jubli Perak Sultan Ahmad Shah, Kuantan Pahang. The leaves of *P. sarmentosum* and *P. nigrum* were oven-dried (45 °C) for five days and then grounded into powder by using a blender machine. The dry leaves were transformed into powder to give higher extraction for an antioxidant compound. The smaller particle size will increase the surface contact between the sample and the extraction solvent (Azwanida, 2015).

2.2 Plant extraction

The extraction method used was maceration method. Solvents which used in this experiment were ethanol and distilled water. The powder of *P. sarmentosum* and *P. nigrum* leaves macerated with a ratio of 1:10 (w/v) by using 95% ethanol and distilled water for 6 hours with agitation over the shaker. Then, the maceration solutions were allowed to remain at room temperature for 24 hours. Next, the sample solutions were filtered and replaced with same new solvent and re-macerated. The entire filtrate was obtained and vaporized by vacuum rotary evaporator under reduced pressure. The crude extracts were stored at 4 °C until further use.

2.3 Antioxidant activity determination

Extracts of the plant samples were tested for their antioxidant activity by

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2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical according to the method described with slight modification (Lee et al., 2011). The reduction process from DPPH to DPPH-H was used to determine the antioxidant activity of ethanol and aqueous extract leaves of *P. sarmentosum* and *P. nigrum.* A stock sample solution of 100 mg/mL concentration was prepared by dissolving 400 mg of each plant extract into 4 mL of 99.99% ethanol. Meanwhile, 0.1 mM solution of DPPH in ethanol was prepared by dissolving 0.0019 g DPPH with 50 mL of 99.99% ethanol. Next, a serial dilution from a prepared stock solution of the sample was made. The samples were two-fold serially diluted to seven different concentrations (3.13 to 200 µg/mL) were prepared for each sample. The stock solution of 10 % ascorbic acid as the positive control was prepared by dissolving 200 mg ascorbic acid in 2 mL of 99.99% ethanol. The solution was then prepared into several final concentration by two serial dilutions (3.13 to 200 µg/mL).

Then, 100 µL from each concentration were prepared earlier was added with 100 µL of 0.1 mM DPPH solution in each well. The mixture was shaken and allowed to stand at room temperature for 30 minutes. The absorbance was measured at 517 nm by using a microplate reader. The experiment was done in triplicate. The percentage of DPPH activity was calculated by the using formula as in Equation 1.

Percent of inhibition (%) = [(A blank - A sample) / A blank] x 100% Equation 1

A blank = Absorbance of DPPH solution (containing all the reagents except test sample)

A sample = Absorbance of DPPH solution after adding the sample extract

The antioxidant activity measured in this method was expressed as percentage inhibitory activity. The values were plotted versus concentration of samples to obtain the amount of antioxidant to decrease the initial DPPH concentration by 50% (IC_{50}). The lower the absorbance of the reaction mixture indicates higher free radical activity. The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) (SPSS ver. 23). The *P* values of less than 0.05 were used as statistically significant.

3. Results and Discussion

3.1 Antioxidant determination on *Piper sarmentosum* and *Piper nigrum*

The stable radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) has been used to determine antioxidant capacity in foods, beverages and plant extract. This method is rapid, simple, accurate and inexpensive which is widely used for measurement of free radical scavenging ability of antioxidants and considered as the best method to study antioxidant activity (Perez-Jimenez et al., 2008; Sumazian et al., 2010).

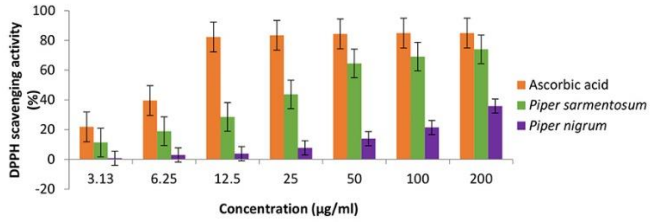

Figure 1: DPPH free radical scavenging activity of ascorbic acid, *P. sarmentosum* and *P. nigrum* of ethanolic extraction

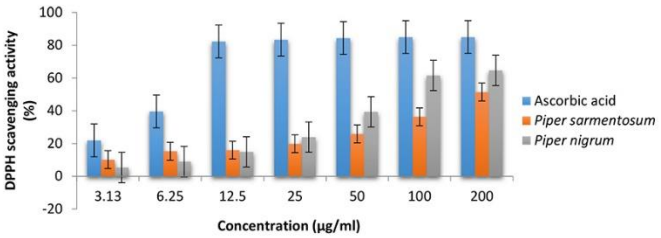

Figure 2: DPPH free radical scavenging activity of ascorbic acid, *P. sarmentosum* and *P. nigrum* of aqueous extraction

Based on Figure 1 and Figure 2, both plant extracts of the leave samples showed antioxidant activity, where the ethanolic extract of *P. sarmentosum* showed a higher percentage of inhibition compared to *P. nigrum*. However, the standard which is ascorbic acid showed higher percentage inhibition compared to *P. sarmentosum*. In Figure 2, *P. sarmentosum* and *P. nigrum* show antioxidant activities. *P. nigrum* shows a higher percentage of inhibition compared to *P. sarmentosum* by using aqueous extraction. However, the standard which is ascorbic acid shows a higher percentage of inhibition than *P. nigrum*.

Throughout the assay, the observation exhibited the colour changes from violet to pale yellow when the concentration of sample increases. DPPH is a stable free radical which will form deep violet colour as it does not disintegrate when mixed with ethanol and methanol solution. The plants extract that contain antioxidant properties can cause the reduction of DPPH, which will cause decolourization of DPPH from violet to pale yellow (Molyneux, 2004). Extracts that have a higher percentage of inhibition have a higher ability in scavenging free radicals. The DPPH free radicals which were scavenged by the plant extracts showed in a concentration-dependent manner.

From the Figure 1, it shows that the ethanol extract of *P. sarmentosum* exhibited the highest radical scavenging activity with 74 %, followed by *P. nigrum* with 35.89 % inhibition at the highest concentration of the sample. On the other hand, the aqueous extract of *P. nigrum* showed the highest radical scavenging activity with 64.68 %, followed by *P. sarmentosum* with 51.42 % inhibition in the highest sample concentration. There was a significant difference in antioxidant activity between *P. sarmentosum* and *P. nigrum* with different extraction method ($p < 0.05$).

Based on the result, the different species showed different antioxidant activities by using different solvent extraction. Ethanolic extraction of *P. sarmentosum* showed higher antioxidant activity compared than aqueous extraction. This finding was supported by the research conducted (Hussain et al., 2009). Ethanol has an intermediate polarity that can extract both polar and non-polar antioxidant compound compare to aqueous solvents such as polyphenol, flavonoid and terpenoid. According to a study, ethanol is preferred to be the best compounds extraction solvent as it is less toxic and effective in extracting phenolic compounds (Karadeniz et al., 2005).

Apart from that, the *P. nigrum* leaves antioxidant activity by using aqueous extraction was higher than using ethanolic extraction because the aqueous solvent is a polar solvent which can extract the polar compound. Pepper leaves contain a polar compound that exhibits antioxidant properties such as phenols, tannins, alkaloids and saponin. The aqueous extraction of *P. nigrum* leaves was well described (Shanmugapriya et al., 2012). The research compared three different solvents to extract antioxidant compounds from *P. nigrum* leaves and found that the most suitable solvent was by using ethyl acetate followed by acetone and aqueous. However, there is a lack of research about the antioxidant activity of *P. nigrum* leaves by using ethanolic extraction due to the sole focus *P. nigrum* fruits.

3.2 IC_{50} value of DPPH Assay

The IC_{50} value of positive control and plant extract was determined by linear and non-linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the sample. Table 2 reported on the IC_{50} value in ethanol, and aqueous extraction of *P. sarmentosum* and *P. nigrum* leaves.

Table 2: IC_{50} values of *Piper sarmentosum* and *Piper nigrum* in different solvents extract

Solvents	IC_{50} value (µg/mL)	
	Piper sarmentosum	***Piper nigrum***
Ethanol	35.18	272.6
Water	182.82	79.89

The IC_{50} of aqueous extract for *P. nigrum* was lower than *P. sarmentosum* with 79.89 µg/mL and 182.82 µg/mL, respectively. Classification based on records that extracts which possess IC_{50} values ranging from 50 to 100 µg/mL is considered to have intermediate antioxidant activity (Phongpaichit et al., 2007). Meanwhile, for extracts with IC_{50} values ranging between 10 to 50 µg/mL are considered as strong antioxidant activity. The extracts with IC_{50} value range more than 100 µg/mL; they are classified as a weak antioxidant. Therefore, this study concludes that the ethanolic extract of *P. sarmentosum* possesses strong antioxidant activity and aqueous extract of *P. nigrum* exhibited moderate antioxidant activity.

From the result of the percentage of inhibition and IC_{50}, there were similarities in the result obtained by measuring the free radical scavenging

activity of *P. sarmentosum,* and *P. nigrum* leaves. The percentage of inhibition of both results and IC_{50} showed that ascorbic acid standard exhibited powerful antioxidant compared to *P. sarmentosum* and *P. nigrum* leaves extract. The ethanolic extraction of *P. sarmentosum* and aqueous extraction of *P. nigrum* showed higher antioxidant activity compared to other methods.

According to a study, smaller IC_{50} possesses higher capability in scavenging free radicals (Jadid et al., 2016). The ethanolic extract of *P. sarmentosum* exhibited significant activity with a low IC_{50} value in comparison with *P. nigrum,* which were 35.18 µg/mL and 272.6 µg/mL, respectively. IC_{50} of the ascorbic acid standard was 6.86 µg/mL, and it was a smaller value compared to *P. sarmentosum* and *P. nigrum* with different extraction method.

4. Conclusion

This study concludes that the plant leaf extracts of *P. sarmentosum* and *P. nigrum* have potent antioxidant activity. There was a significant difference between two species with different solvents for extraction. Ethanolic extraction is the best solvent to extract *P. sarmentosum* while aqueous extraction was found to be a suitable solvent to extract *P. nigrum* to obtain the compounds with best antioxidant activity. The research result indicates that the choice of extraction solvent to obtain beneficial compounds is crucial.

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