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Research Article

EFFECT OF HEATING AT 95 °C ON ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT IN EXTRACTS OF PLECTRANTHUS AMBOINICUS LEAVES

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ABSTRACT

Nowadays, many of antioxidants on the market, but most of the antioxidant is heat sensitive which their antioxidant capacity is decreased during processing. So, a heat stable antioxidant is important to ensure their effectiveness for neutralize the free radical in the human body. The aim of this study is to determine a presence of heat stable antioxidant activity of *Plectranthus amboinicus* leaves. Dried-powdered leaves was soaked water and heated at 95 °C for 0, 30, 60, 90 and 120 minutes, respectively. Water extracts were freeze dried to obtain the powdered water extracts. The remaining of leave's biomass was sequential extracted with ethanol and ethyl acetate to obtain ethanol and ethyl acetate extract. The antioxidant activity and total phenolic content of the extracts were determined by 2,2-diphenyl-1-picrylhydrazyl and Follin Ciocalteau assay, respectively. The results were shown that the ethanol extract had the highest ($p \leq 0.05$) antioxidant activity compared to the water and ethyl acetate extract. The ethanol extract had also the highest phenolic content (77.99 ± 0.52 GAE $\mu\text{g}/0.5$ mg extract). It can be concluded that the leaves of *P.amboinicus* might contained a heat stable antioxidant substance which contributed by phenolic substances.

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INTRODUCTION

Currently, more than hundred phytochemical substances have been discovered and proven scientifically to exhibit antioxidant activity (Azzini *et al.*, 2017). These phytochemicals are including curcumin from *Curcuma longa*, carotenes and carotenoid from *Daucuscarota*, polyphenols (ellagic acid, gallic acid and tannins) from *Embilica officinalis*, glycyrrhizin from *Glycyrrhizaglabra*, cetachin from *Cameliasenensis* (Sulaiman *et al.*, 2013). Major application of the antioxidants is included as health supplements, active ingredient in cosmetic products and food preservatives (Sindhi *et al.*, 2013). However, most of discovered and developed antioxidants reduce their antioxidant activity after repetition exposure to heat above 60 °C during multi-step processes and value-chain distribution. Thus, it is important to discover the heat-stable antioxidant substances which able to withstand under such condition. In this attempt, we have explored the heat-stable antioxidant extract from *Plectranthus amboinicus*

(Lamiaceae) or locally known as “pokok bangun-bangun” or “ati-ati hijau”. Although many considerable works on the antioxidant activity of this particular plant are conducted, but no study on the heat stability of the extract (Azman *et al.*, 2015; Gupta *et al.*, 2013).

MATERIALS AND METHODS

Sample

Twenty plants of *P. amboinicus* were cultivated at herbal garden of Universiti Malaysia Kelantan, Campus Jeli, Jeli, Kelantan, Malaysia on Jun 2016 and the leaves were collected on February 2017. All leaves were washed with tap water and dried in electric oven at 65 °C for 2 hours and followed by drying at 37 °C for two days. The dried leaves were ground into powder by using electrical grinder.

Heat treatment and extraction

The in-house extraction method was applied for extraction of powdered leaves of *P. amboinicus*. Briefly, five glass tubes

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were labelled with A, B, C, D and E. All of the tubes were filled with 5 grams of powdered leaves and filled with 40 ml distilled water. All of tubes were sonicated in ultrasonic water bath for 30 minutes. Tube A was kept at room temperature and re-labelled as time 0. While tubes B, C, D and E were transferred into the pre-heated water bath at 95 °C. Tube B, C, D and E were removed from water bath at 30, 60, 90 and 120 minutes, respectively. All tubes were centrifuged at 1000 g for 30 minutes at 10 °C and supernatants were freeze dried to produce the water extracts. The remaining biomass of tubes were further extracted with 40 ml ethanol in an ultrasonic water bath for 30 minutes and were repeated with a fresh ethanol for 2 times. All of the filtered extracts were dried under reduced pressure using a rotary evaporator at 40 °C and produced the ethanol extracts. Then, the remaining biomass from all tubes were further extracted with 40 ml ethyl acetate using the ultrasonic water bath for 30 minutes and repeated with a fresh ethyl acetate for 2 times. All of the filtered extracts were dried under reduced pressure using a rotary evaporator at 40 °C and produced the ethyl acetate extracts. All of the extracts were kept on -20 °C until used for assays.

2,2-diphenyl-1-picrylhydrazyl (DPPH) free Radical Scavenging Assay

The DPPH free radical assay was followed the method as previously described (Abdullah *et al.*, 2013). Extracts were dissolved in 100 % dimethyl sulfoxide (DMSO). Then, further diluted into five concentrations, viz., 6.13, 15.5, 25, 50 and 100 µg/ml in 100% ethanol and final concentration of DMSO was 1 %. The solution of 0.004 % DPPH was prepared in 100% ethanol and 1.0 ml of DPPH solution was mixed with 1.0 ml of the extracts. The reaction mixture was vortex thoroughly and left in the dark at room temperature. After 30 min, optical density (OD) of the mixture was measured at 517 nm. Ascorbic acid was used as positive control. The ability to scavenge DPPH radical was calculated as follows: % of DPPH free radical scavenging activity = $(OD_{\text{control}} - OD_{\text{sample}}) / OD_{\text{control}} \times 100$ %. The amount of antioxidant necessary to decrease the initial DPPH concentration by 50 % (IC₅₀ value) was calculated from the graph concentration of sample versus % of DPPH free radical scavenging activity.

Total phenolic content

Total phenolic content of the extracts was determined using Folin-Ciocalteu assay (Abdullah *et al.*, 2013). Briefly, all extracts were diluted to the concentration of 0.5 mg/ml to obtain the readings within the standard curve of the range of 6.125 µg/ml to 100 µg/ml of gallic acid. A solution of 0.5 ml extracts (0.5 mg/ml) were mixed with 1.5 ml of 10 % (v/v) Folin-Ciocalteu reagent. The mixtures were incubated at room temperature for 5 minutes. After addition of 2 ml 7.5 % (w/v) of sodium carbonate, the mixture was incubated in dark for one hour. The absorbance of a blue color that developed was measured at 765 nm by using a spectrophotometer. The absorbance values were then compared with gallic acid standard curve. The assay was carried out in triplicates and repeated for three times. Total phenolic content of samples were expressed as µg gallic acid equivalent (GAE)/ 0.5 mg of extract.

Statistical Analysis

The experiment's data were reported as means ± standard deviations. Significant differences at $P \leq 0.05$ among the means from triplicate samples were determined by t-test and ANOVA.

RESULTS AND DISCUSSION

This study was conducted to investigate the effect of heat treatment on antioxidant and total phenolic content (TPC) in leaves of *P. amboinicus*. The evaluation of antioxidant activity of extracts by DPPH assay were presented as IC₅₀ value (Figure 1). The IC₅₀ for water, ethanol and ethyl acetate extracts were ranged from 148.80 to 225.43, 11.76 to 36.05 and 61.35 to 110.56 µg/ml, respectively. Results clearly show that antioxidant in ethanol extracts were scavenged free radical more effective than ethyl acetate and water extracts. The high antioxidant activity in ethanol extracts might be due to the presence of phenolic compounds (TPC) as showed in Figure 2. Where, the TPC in water, ethanol and ethyl acetate extracts were ranged from 8.17 to 35.60, 65.67 to 77.99 and 24.84 to 49.56 µg GAE/ 0.5 mg of extract, respectively. The correlation analysis between IC₅₀ and TPC of the extracts were shown a positive association ($r = 0.745$ to 0.923). This correlation was indicated that the phenolic compounds in extracts might be responsible for antioxidant activity. Previous studied were reported the presence of phenolic compounds in *P. amboinicus* with antioxidant activity (Andarwulan *et al.*, 2014; Bhatt and Negi, 2012; Bhatt *et al.*, 2013). These compounds include caffeic acid, rosmarinic acid, coumaric acid, quercetin, rutin, coumaric and gallic acid (Gupta *et al.*, 2013).

Results were also indicated that phenolic substances less extracted in water but more extracted by ethanol. However, ethanol was unable to extract all of phenolic in plant biomass as shown by presence of phenolic in ethyl acetate extract. This observation may due to a varying polarity of phenolic compounds in the leaves as described by several authors when extracted phenolic compounds from plants (Anokwuru *et al.*, 2011; Do *et al.*, 2014; Kallithraka, *et al.* 1995). In water, ethanol and ethyl acetate extracts, the heat treatment for 30, 60, 90 and 120 minutes were significantly ($p \leq 0.05$) decreased the IC₅₀ compared to untreated (0 minute). Low IC₅₀ indicates a strong antioxidant activity. This result was in agreement with several researchers that studies on an effect of heat towards antioxidant activity in several plants extract such as eggplant (Arkoub-Djermoune *et al.*, 2016), chilli pepper (Shaimaa *et al.*, 2016), saffron honey (Nayik and Nanda, 2016), citrus peels (Jeong *et al.*, 2004), biodiesel from *Jatropha curcas* (Silva *et al.*, 2013) and chokeberry (Cristea, 2016). The reason for the increase of antioxidant activity in heat-treated sample compare to untreated is due to a food matrix effect which act a barrier to heat effect or induce the degradation antioxidant compounds (Barriuso *et al.*, 2016; Raikos, 2017). Moreover, a formation of Maillard products which resulted from degradation of phenolic compounds during thermal treatment have been observed in several experiments where these products showed the significantly higher antioxidant activity than the initial phenolic compounds (Tamanna and Mahmood, 2015; Buchner *et al.*, 2006; Murakami *et al.*, 2004).

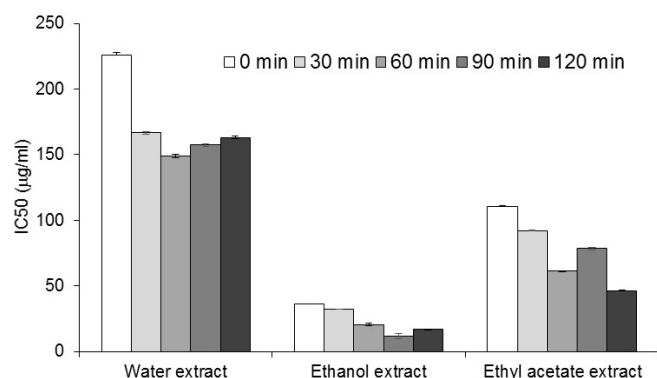


Figure 1 Antioxidant activity of the heat-treated *Plectranthus amboinicus* leaves extracts by DPPH assay.

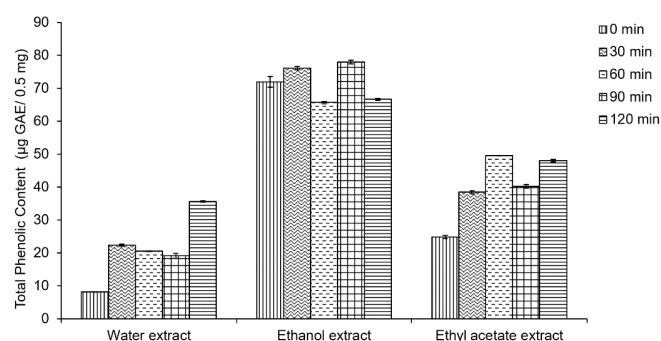


Figure 2 Total Phenolic Content (TPC) in the heat-treated *Plectranthus amboinicus* leaves extracts.

CONCLUSION

In conclusion, the study was demonstrated that the leaves of *P. amboinicus* was contained a heat stable antioxidant substance which might contributed by phenolic compound(s).

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