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# ANTIOXIDANT ACTIVITY AND HPLC ANALYSIS OF EXTRACT FROM *AMPELOCISSUS CINNAMOMEA (VITACEAE)* TUBER

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## ABSTRACT

In the ongoing quest for a powerful antioxidant to neutralize free radicals, we investigate the potential antioxidant activity of the tuber of *Ampelocissus cinnamomea* (Vitaceae). The tuber was used to increase the endurance of the Temiar ethnicity. The objectives of this study were to determine antioxidant activity, total phenolic content (TPC), total flavonoid content (TFC), and to provide a HPLC chromatogram of the tuber extracts. The powdered tuber was extracted consecutively with petroleum ether, ethyl acetate and methanol to produce petroleum ether (EP) extract, ethyl acetate (EA) extract and methanol (ME) extract. The antioxidant activity, TPC and total TFC in the extracts were measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteau and aluminium chloride, respectively. The extract profile was identified by the HPLC. The concentration of PE, EE and ME that scavenge 50% of DPPH free radicals ($IC_{50}$) were ≥ 100, 12.52 ± 2.53 and 20.37 ± 3.12 µg/ml, respectively. The TPC in PE, EE and ME were 13.333 ± 3.055, 713.894 ± 28.666 and 708.439 ± 17.082 µg gallic acid equivalent/mg extract, respectively. The TFC in PE, EE and ME were 3.23 ± 1.33, 99.51 ± 3.19 and 115.53 ± 4.46 µg quercetin equivalent /mg extract, respectively. The HPLC chromatogram of the extracts revealed several major peaks that their UV-Vis spectrum resembles the class of flavonoids. In conclusion, the results were suggested to investigate in more detail an antioxidant potential in several antioxidant testing systems and isolate the active compound or compounds.

Keywords: *Ampelocissus cinnamomea*, tuber, methanol, antioxidant.

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## INTRODUCTION

Free radicals are atoms or molecules that have unpaired electrons, usually unstable and highly reactive (Alkadi 2020) Oxygen free radicals or reactive oxygen species, such as superoxide, hydroxyl radicals, and peroxyl radicals, hydrogen peroxide, hypochlorous acid and ozone, are generated during the metabolism process of oxygen as well as due to consumption on alcohol, drugs, environmental pollutant, temperature and food. Reactive oxygen species can initiate lipid peroxidation, cause DNA strand breaks, and unstoppable oxidize all molecules in biological membranes which resulting in cellular pathology such as premature aging, liver disease and cancer (Di Meo & Venditti 2020)

Although the body system has its own defense abilities to neutralize reactive oxygen species, in some circumstances it does not function as expected and this may be due to illness and personal lifestyle. Various proofs have shown that an oral consumption of exogenous antioxidants comes mainly from vegetable sources can restore its function. Several plants extract, standardized extracts, partially purified extract, and pure compound have been commercially available as hepatoprotective agent against reactive oxygen species. These substances are including silymarin derived from milk thistle (Fallah et al. 2020) and, Liv.52 (Himalaya®) contains *Capparis spinosa*, *Cichorium intybus*, *Terminalia arjuna*, *Cassia occidentalis*, *Achillea millefolium* and *Tamarix gallica* (Yildirim et al. 2022)

*Ampelocissus* cinnamomea is a climbing species native to Malaysia, Singapore and Indonesia (Kusuma 2014, Ng 2014). This species is known locally as "ubi nyaru", "ubi spring" or "ubi sembelit tujuh", "akar jari biawak", "akar keladi enggang" and "gadung samak. Traditionally, a poultice of crushed leaves is placed over the wound, abdomen during labor or sore legs. Decoction of its tuber is used to treat hypertension, gout, reduce cholesterol as well as a liver tonic (Mahathir 2002)). However, there are no scientific report on antioxidants and phytochemicals of the species. Thus, this study was conducted to determine antioxidant activity and phytochemical of the wild tuber of *Ampelocissus cinnamomea.*

## METHODS

### Plant material

Tubers of wild *Ampelocissus cinnamomaea* were collected from Air Banun, Grik, Perak. The plant specimen was checked by the botanist. The tuber was thoroughly washed, and the skin peeled off. The peeled tuber was cut into slices, crushed with pastel and mortar until it becomes a paste. The tuber paste was dried in electric oven for 4 days at 40°C. Dried sample was kept in glass bottle at 4°C.

### Extraction

The extraction was followed the method described by Nawaz *et al.* (2020) with some modification. Two hundred grams of dried tuber biomass were filled into a 2000 ml glass bottle containing 400 ml 100% petroleum ether and sonicated for 30 minutes in an ultrasonic water bath with 27oC. The extract was filtered with Whatman Filter Paper #.1. That process was repeated six times. The tuber biomass was extracted six times by ethyl acetate and ten times by methanol. The respective solvents were removed under reduction pressure with rotating evaporator to produce a crude solid extract of petroleum ether (PEE), ethyl acetate (EAE) and methanol (ME). The extract samples were stored in an airtight glass bottle at -20ºC until they were used for testing.

### Preparation of stock and working solution of sample, positive control, and standard

The stock solution of PEE, EAE, ME, ascorbic acid, gallic acid and quercetin were prepared in 100% DMSO to give the concentration of 100 µg/ml.

### DPPH Assay

The DPPH assay followed the methods as described by Truong *et al*., (2019) with some modification. The 0.1 mM of DPPH solution was prepared in methanol. Six concentrations of FE, EAE, ME and ascorbic acid, i.e., 3.125, 6.25, 12.5, 25, 50 and 100 µg/ml were prepared in 100% DMSO. Then, 50 µl of the respective concentration of the sample was mixed with 950 µl methanol and 1000 µl of 0.1 mM of DPPH solution. The control was made by mixed 50 µl DMSO, 950 µl methanol and 1000 µl of 0.1 mM DPPH solution. All the mixtures were incubated in a dark room for 30 minutes and then transferred into 1.5 ml plastic cuvette. The absorbance was read at 517 nm by using spectrometer. The scavenging

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activity was calculated according to the formula below: Scavenging activity (%), A = [ ($A_o$ - $A_e$) ÷ $A_o$] x 100. A is a percent reduction of DPPH, $A_o$ is a control absorbance unit, and $A_e$ is a sample absorbance unit. The DPPH scavenging capacity of sample was presented as $IC_{50}$ value, a free radical scavenging efficiencies This value is referred to the concentration of sample to scavenge 50% of DPPH free radicals which calculated from a graph of concentration of sample against the percentage of scavenging activity.

**Total phenolic content**

Total phenolic content (TPC) in the PE, EE and ME was determined by Folin – Ciocalteau assay (Truong *et al*., 2019). Sample and Gallic acid were prepared in 100% of DMSO. The concentration of samples was 100 µg/ml, and the seven concentrations of Gallic acid were 800. 400, 200, 100, 50, 25, and 12.5 µg/ml. Reagent without extract (only 100% DMSO) was used as a control. Then, the 200 µl of sample or standard were mixed with 1000 µl of 10% Folin-Ciocalteu reagent. After that, the mixtures were standing for 5 minutes, followed by 800 µl of 2% sodium bicarbonate. The mixture was incubated for two hours in the dark. Then, the absorbance of the blue colour that developed was measured at 765nm using an UV-VL spectrophotometer (Spectroquant Pharo 300, MERCK). The experiments were carried out in triplicates. The total phenolic content calculated by using linear regression of concentration of Gallic acid versus absorbance value at 765 nm and the values were expressed as µg Gallic acid equivalent (GAE) /mg extract.

**Total Flavonoid Content**

Briefly, the mixture of 2000 µl extract at 100 µg/ml or quercetin (reference standard) at various concentration, (i.e., 50, 100, 200, 400, 800, 1600 µg/ml) and 500 µl of 5% $AlCl_3$ and 500 µl of 1M potassium acetate solution were incubated at room temperature for 15 min. DMSO (100%) was used as a control. The absorbance of all samples was then measured at 415nm using a 506 nm using UV-VL spectrophotometer (Spectroquant Pharo 300, MERCK). Total flavonoid content (TFC) was shown as mg of quercetin equivalent (QE) per mg of extract.

**High Performance Liquid Chromatography (HPLC)**

Ten milligram of extract was dissolved in 1ml mobile phase (70% acetonitrile in water) and filtered. Then, 20 µ of sample solution was injected into Shimadzu Prominence HPLC System with SPD-M20A DAD which consist of Cosmosli $C_{18}$ column (250 mm x 4.6 mm). The mobile phase was acetonitrile-water (70:30 v/v) with run with isocratic mode at 1.0 ml/min.

**RESULTS AND DISCUSSION**

This study was the first report to examine the antioxidant activity of Ampelocissus cinnamomaea tuber extract. Only one study of this species related to antioxidant by Kusuma (2014) which reported the antioxidant activity of ethanol, ethyl acetate and n-hexane leaf extract using a DPPH assay. Yet the study is superficial.

In this study, the antioxidative activities of the tuber extract of Ampelocissus cinnamomea, i.e., PE, EE and ME were determined through the free radical scavenging activity via their reaction with the stable DPPH radicals (Figure 1). Results showed that the scavenging activity of samples on DPPH radical was positively correlated with the concentration. The correlation coefficients for PE, EE and EM were 0.9634, 0.7764 and 0.8220, respectively. The free radical scavenging efficiencies of extracts was showed in the TABLE 1. EE was the most potent extract with an $IC_{50}$ value of 12.54 ± 0.56 µg/ml. The ME and PE exhibited a lower radical scavenging activity with respective $IC_{50}$ values of 20.83 ± 0.74 µg/mL and more than 100 µg/ml. A low $IC_{50}$ indicates that the extract has a high ability to function as a DPPH scavenger. A high $IC_{50}$ indicates low scavenger salvage activity as more scavengers were needed to achieve a 50% salvage reaction.

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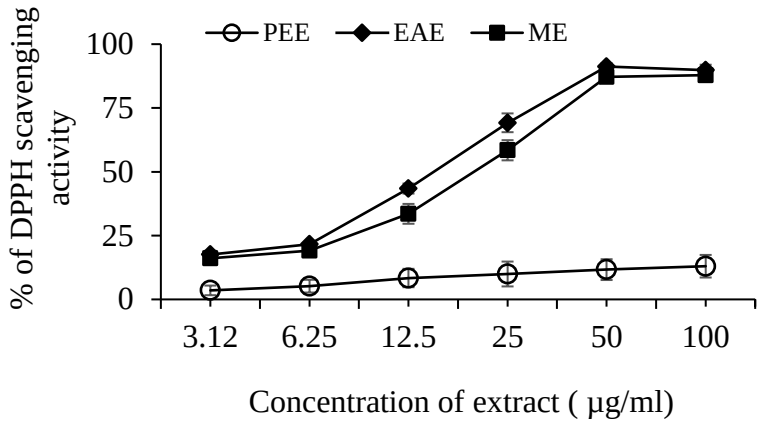


Figure 1: DPPH radical scavenging activity of extracts

Table 1 shows the TPC and TFC of the PE, EE, and ME. The ME showed the highest amount of TPC and TFC compered to EE and PE. There was a positive correlation (r>0.9) between TPC and TFC of the sample. The TPC and TFC showed the strong corelation (r> -0.9) with the free radical scavenging efficiencies, i.e., $IC_{50}$ value of DPPH assay. Thus, the phenolic components of extracts may be the main contributors of antioxidant activity in the neutralization of free radicals. Antioxidant activity of phenolic and flavonoid compounds derived from plants has been reported elsewhere (reviewed in Gulcin, 2020; Kiokias et al. 2020; Swallah et al. 2020)

**Table 1: DPPH $IC_{50}$, total phenolic content (TPC) and total flavonoid content (TFC) of *Ampelocissus cinnamomea* tuber extract**

| Assays | Extract | | | Ascorbic acid |
|---|---|---|---|---|
| | PEE | EAE | ME | |
| DPPH $IC_{50}$ (µg/ml) | >100 | 12.54 ± 0.56 | 20.83 ± 0.74 | 15.71 ± 0.48 |
| TPC (µg GAE/mg extract) | 13.33 ± 3.06 | 713.89 ± 8.66 | 708.44 ±7.27 | - |
| TFC (µg QE/mg extract) | 3.33 ± 1.33 | 99.33 ± 3.19 | 115.53 ± 4.46 | - |

Figure 2 shows the HPLC chromatogram of PE, EA and ME at 254nm. Under these HPLC conditions, the number of major spikes for PE, EA and ME was 13, 12 and 13. This suggested that the tuber contains non-polar, polar and polar secondary metabolites. In the sample of EE and ME, the several peaks show (Data not showed) the UV-VIS spectral data were resemble to the flavonoid as detected in grape leaves (REF). However, further investigation is required to confirm the class of secondary metabolites.

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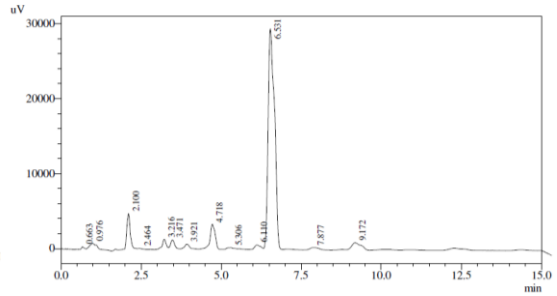
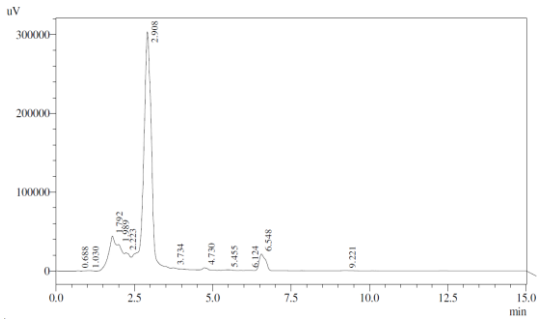
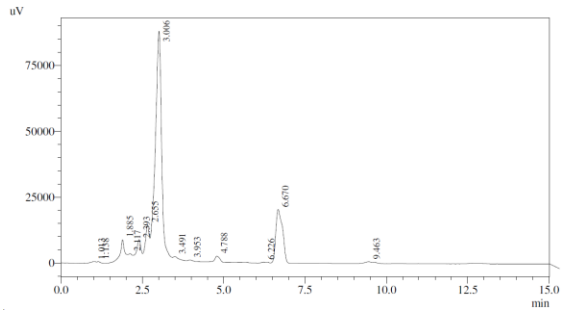



Petroleum ethe extract (PE)




Ethyl acetate extract (EE)




Methanol extract (ME)

Figure 2: HPLC chromatogram of of *Ampelocissus cinnamomea* tuber extract.

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