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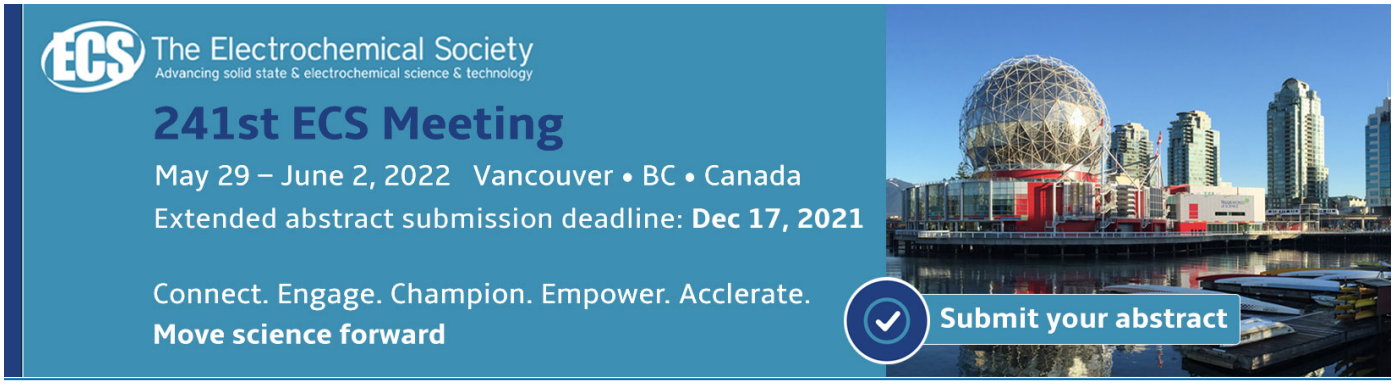

Elucidation of total phenolic content and antioxidant activity in medicinal Aroid, *Alocasia longiloba* Miq.

M Nur-Izzati[1], M Arifullah[2], A A Nazahatul[3], S Klaiklay[4], P Chumkaew[5], M Z Norhazlini[3], F Abdulhafiz[2] and H Zulhazman[3*]

[1]Prisma Laboratory (M) Sdn. Bhd., No.36-01 & 38-01, Jalan Kempas Indah 1/1, Taman Kempas Indah, 81300 Johor Bahru, Johor, Malaysia.
[2]Faculty of Agro Based Industry, Campus Jeli Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia.
[3]Department of Natural Resources & Sustainability Science, Faculty of Earth Science, Campus Jeli Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia.
[4]Department of Chemistry and Centre of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkla 90112, Thailand.
[5]Faculty of Science and Industrial Technology, Prince of Songkla University, Suratthani Campus, Suratthani 84000, Thailand.

*E-mail: zulhazman@umk.edu.my

Abstract. *Alocasia longiloba* Miq belongs to Araceae family, believed to have the medicinal potential. However, the scientific evidences on this plant were limited. Therefore, this study aimed to evaluate the total phenolic content (TPC) and antioxidant activity of the different extracts (methanol, ethyl acetate and hexane) of *A. longiloba* leaf blades. The TPC was investigated by Folin-Ciocalteu method and antioxidant activity was determined by DPPH scavenging assay. The results showed ethyl acetate extract had the highest content of phenolic with 46.013 mg GAE/g followed by methanol extract (32.936 mg GAE/g) and hexane extract (31.782 mg GAE/g). The hexane extract exhibited the highest DPPH antioxidant acitivity, followed by ethyl acetate and methanol with IC_{50} values 2.519 µg/mL, 2.758 µg/mL and 9.542 µg/mL respectively. The results indicate *A. longiloba* has natural sources of antioxidant that can be used for the medicinal purposes.

1. Introduction

Araceae and sometimes regard as Aroid, consists of 105 genera and more than 3, 300 species had been identified. The plant is a herbaceous and diverse in its morphology, displaying attractive leaf make them a well-known recognized feature by the scientists [1, 2, 3]. The active ingredients from plants possibly as valuable source in maintaining human health, where about 80 % of individuals preferred to consume medicinal herbs [4, 5, 6]. The presence of alkaloids, phenolics, triterpenoids and tannins were the common bioactive compounds found in plants where each of them have diverse potent activities [7, 8].

[9] reported *Anthurium schott* and *Philodendron schott* were used as an effective medicinal herbs in treating headaches, malaria, fevers and liver problem, whereas *Aglaonema treubii* have the

bioactive compound potentially to cure diabetes type 2 and HIV-1 infection [3]. Another reports on the use of Aroids as medicinal herbs is by [10], he stated *Colocasia esculenta* believed to heal cut and injuries by using its leaves and tubers.

Other than used as medicinal herbs, Aroids also edible to be consumed by human and animals. *Xanthosoma* (i.e. *X. sagittifolium*) and *Colocasia* (i.e. *C. esculenta*) are the most popular genera among the locals and was consumed as food [11]. However, improper food preparation will cause food poisonous due to presence of needle-like raphids of calcium oxalate, biological reaction causing inflammation on mouth, lips and throat [12].

Besides, Araceae are widely used as ornamental plants due to their attractiveness of leaf foliage [11]. [13] listed *Aglaonema*, *Dieffenbachia*, *Philodendron* and *Syngonium* are the examples of ornamental foliage plants, widely and commonly sell at plant nusery, helps locals to generate income.

"Keladi Candik" is the local names of *Alocasia longiloba* Miq which belongs to Araceae family. The species is native in Peninsular Malaysia, China, Laos, Thailand, Borneo, Sumatera and Sulawesi [14]. According to [15], *A. longiloba* is similar to *A. lowii*, *A. denudate*, *A. veitchii*, *A. singaporensis* and *Caladium veitchii*. The plant traditionally used by locals to treat the wounds [16]. Scientific studies on the chemical properties of this plant genus are lacking. Therefore, we aimed to investigate the chemical properties of *A. longiloba* by evaluating the total phenolic content (TPC) and antioxidant activity of the sample extracted in different organic solvents.

2. Materials and Methods

2.1. Plant materials and extraction

The samples of *A. longiloba* were collected from Kampung Tiong Dalam, Kota Bharu, Kelantan, Malaysia. The plant materials (leaf blades) were chopped to small pieces and dried in the oven for 3 days at 50 ℃. Next, the plant materials were ground using mechanical blender till the samples become fine powder.

The plant extraction were conducted based on [17], reflux extraction protocol with slightly modification. 3.5 g of fine powder were diluted into 45 mL of extraction solvent; methanol, ethyl acetate and hexane. The diluted samples were heated for 4 h with temperature between 50 to 70 ℃. Next, the obtained solutions were allowed to cool at room temperature. The extracts were filtered and concentrated using rotary evaporator (Heildolph, Germany) until all the solvent evaporated.

2.2. Chemicals

2-diphenyl-1-picrylhydrazyl (DPPH), Folin ciocalteu's reagent, sodium bicarbonate, gallic acid, and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Organic solvents (AR grade)- absolute ethanol, methanol, ethyl acetate, and hexane were from HmbG (Orioner Hightech Sdn.Bhd, M'sia). Water was deionized and purified by Milli-Q system.

2.3. Determination of total phenolic content (TPC)

Total phenolic content *A. longiloba* extract were conducted using Folin-Ciocalteu reagent described by [18] and [19] with a slightly modifications. Briefly, a 1.0 mg of the ethanolic crude extract was dissolved in 1 mL of distilled water (1 mg mL-1) and vortex till becomes homogenous stock solution. Next, 200 µL of diluted extract mixed with 3 mL of dionized water. Then, the Folin-Ciocalteu reagent was added to the solution. After 3 min of incubation, 20 % (w/v) sodium carbonate was added to the solution and allowed to stand for 1 h at room temperature before the absorbance was measured at 765 nm using UV/vis spectromphotometer (FLUOstar® Omega, Germany). The contents of total phenolics were calculated using a calibration curve from gallic acid standard solution and expressed as mg gallic acid equivalents (GAE) per gram of sample (mg/g). All the test were conducted in triplicates.

2.4. Determination of Antioxidant Activity (DPPH scavenging assay)

The DPPH assay was carried out based on method described by [20] and [21] with modifications to adapt with the samples. Briefly, 2.5 mL of the extract solution was prepared by dissolving different

amounts of 300, 350, 400, 450 and 500 µg/mL extract respectively in 2.5 mL of DPPH solution in a test tube. Each sample were prepared in triplicates. After 20 min of incubation, the reading of scavenging effect was measured using UV/vis spectromphotometer (FLUOstar® Omega, Germany) at 517 nm. The DPPH scavenging activity was calculated using the following equation:

DPPH scavenging activity (%) = [(Abs control – Abs extract)/ Abs control] x 100 (1)

Where:

Abscontrol = Absorbance of DPPH + absolute ethanol

Abssample = Absorbance of DPPH radical + sample or standard

The percentage of scavenging activity was recorded and plotted as y-axis and concentration of extract and ascorbic acid as x-axis. The 50 % of inhibition (IC_{50}) was determined referring to the graph plotted.

2.5. Statistical analysis

All test were carried out in triplicates. The data were analysed using spreadsheet software [22] and were presented in mean ± standard deviation.

3. Results and Discussion

Based on the study conducted, the total extraction yield (%) of *A. longiloba* with methanol, ethyl acetate and hexane solvent showed 3.5 g of fine powder has produced approximately 24 %, 3.14 % and 2% of yields respectively.

3.1. Total phenolic content (TPC)

Total phenolic contents in the different extract solvent of *A. longiloba* are shown in Table 1. Ethyl acetate extract has the highest content of phenolic followed by methanol and hexane extract with the value 46.0125±0.0010, 32.9362±0.0006 and 31.7823±0.0006 mg GAE/g) respectively. This indicates ethyl acetate as the polar solvent able to extract the phenolic compounds in *A. longiloba* efficiently. From the data obtained, the phenolic content in methanolic extract lower than ethyl acetate eventhough the methanol has high polarity. [23] explained the phenolic content is depend on their chemical reducing capacity relative to Gallic acid, not just through the measurement of phenolic compounds. According to [24], the presence of total phenolic compounds in an extract is not specific to polyphenols. The other compounds could be reacted with the Folin- ciocalteu's reagent, caused the increasing of phenolic concentration in an extract. In addition, many types of phenolic compounds react differently in this method as it depends on the number of phenolic groups they have.

Table 1. The total phenolic content of *A. longiloba* in different solvents.

Solvent	Total phenolic content (mg GAE g^{-1})
Methanol	32.9362±0.0006
Ethyl acetate	46.0125±0.0010
Hexane	31.7823±0.0006

Values are expressed as mean±standard deviation.

[25] stated the phenolic compounds in the plant believed had potent antioxidant property, thus potentially act as natural remedies to treat illness. The phenolics capable to be radical scavengers and metal chelators [26]. Therefore, the presence of the phenolics compound in all the extracts indicates *A. longiloba* has the properties to be used as the medicinal herbs.

3.2. Antioxidant Activity

The range of radical scavenging activity from three different extracts were between 2.4823±0.0006 % to 77.4232±0.0012 % (Table 2). Apart from that, the values of DPPH radical scavenging activity were inversely proportional with absorbance values as the percentage of DPPH radical scavenging activity was increased when the absorbance was decreased. Whereas the scavenging activity were directly proportional to the concentration of the solution.

The methanol fraction of plant extract showed the lowest antioxidant activity which were 2. 4823±0.0006 %, 10.4019±0.0012 %, 19.9764±0.0010 %, 21.7494±0.0026 %, and 24.5863±0.0020 % in five different of concentrations which were 300, 350, 400, 450 and 500 µg/mL respectively. However, hexane fraction had the highest percentage of radical scavenging activity which were 56.8558±0.0026 %, 64.4208±0.0006 %, 75.2955±0.0012 %, 73.2861±0.0012 %, and 77.4232±0.0012 % respectively in different concentrations. While for the ethyl acetate fraction, the percentage of radical scavenging activity was higher than methanol and lower than hexane with 54.8463±0.0025 %, 59.5745±0.0015 %, 63.0024±0.0010 %, 67.4941±0.0000 % and 72.8132±0.0006 % in 300, 350, 400, 450 and 500 µg/mL respectively.

Table 2. The DPPH radical scavenging activity of *A. longiloba.*

	*Inhibition of DPPH (%)					
Extract	300 (µg mL^{-1})	350 (µg mL^{-1})	400 (µg mL^{-1})	450 (µg mL^{-1})	500 (µg mL^{-1})	IC_{50} (µg mL^{-1})
Methanol	2.4823±0.0006	10.4019±0.0012	19.9764±0.0010	21.7494±0.0026	24.5863±0.0020	95.42
Ethyl acetate	54.8463±0.0025	59.5745±0.0015	63.0024±0.0010	67.4941±0.0000	72.8132±0.0006	27.58
Hexane	56.8558±0.0026	64.4208±0.0006	75.2955±0.0012	73.2861±0.0012	77.4232±0.0012	25.19

*Mean±SD

From this study, it presented that the extracts of *A. longiloba* were able to decolorize the DPPH from purple to yellow as the free radical scavenging activity of the extracts can be described in order as hexane extract> ethyl acetate extract> methanol extract. Although, methanol can be recognized as the most polar solvent in order to extract the phenolic compound, the antioxidant activity in methanol extract of *A. longiloba* showed the lowest radical scavenging activity. This can be supported by [27], indicated the scavenging activity of methanol extract of *Trifolium pratense* was lower than hexane extract and chloroform extract.

Ethyl acetate extract was the second highest of antioxidant activity, this extract could be known to scavenge free radicals and reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions and singlet oxygen. Furthermore, the ethyl acetate extract was able to extract the phenolic and nitrogenous compounds [28].

Lastly, the hexane extract was the highest antioxidant activity. This statement was supported by [29] which stated that the radical scavenging activity by DPPH of pepper extract was efficient in the non-polar and mid polar solvents as more carotenoids were extracted in hexane extract. It can be said that hexane was the best solvent to extract the antioxidant in *A. longiloba.*

The antioxidant activity of A. longiloba can be expressed as inhibitory concentration (IC50). The IC50 is the concentration of extract that was needed to decrease DPPH radical scavenging activity by 50 %. It can be reached from a calibration curve for the extract. The IC50 value from Figure 1 illustrates as descending order which was Methanol extract > Ethyl acetate extract > Hexane extract > Ascorbic acid. Methanol has the highest IC50 value with the value of concentration 9.542 µg/mL which means that methanol extract has the lowest antioxidant activity. Despite of that, hexane extract has the lowest of IC50 value among these three extract (2.519µg/mL). The ascorbic acid was used as standard for antioxidant activity and it had the lowest IC50 value (2.088 µg/mL) which indicates had the highest antioxidant activity. [30] said that the lower IC50 shows the highest effectiveness of the antioxidant. [28] in her study revealed that ethyl acetate and hexane act as intermediate antioxidant

activity where hexane was commonly used as to extract terpenoid which is bioactive compound in plant. The role of terpenoids were acting as pigment for photosynthesis, ensuring membrane integrity, attracting to pollinators, involved in the protein N-glycosyla. In addition it also play an important role in human health.

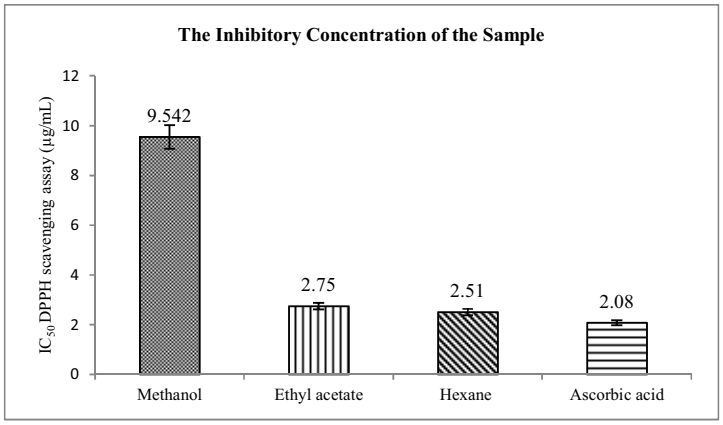

Figure 1. The IC_{50} of DPPH.

4. Conclusions

From the study conducted, it can be concluded that the total phenolic content of *A. longiloba* in ethyl acetate extract was more efficient as it gave the highest value when compared to methanol and hexane extract. The result of the antioxidant activity which expressed by the percentage of free radical scavenging activity showed that hexane extract had the highest antioxidant activity and had the lowest inhibitory concentration (IC_{50}) among the other two extract (methanol and ethyl acetate). In the nutshell, *A. longiloba* have the potential of antioxidant that can be used for the medicinal purposes.

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