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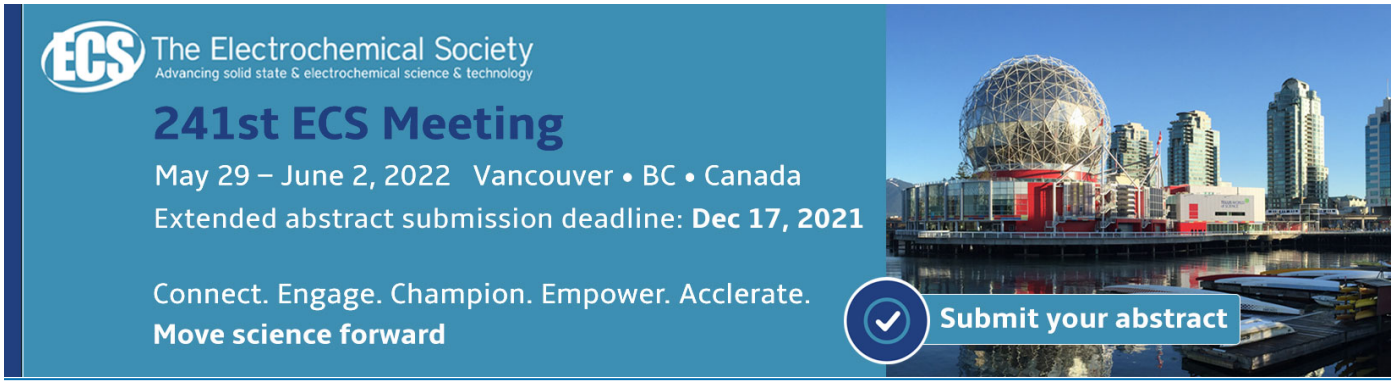

Total phenolic content and antioxidant activity of an edible Aroid, *Colocasia esculenta* (L.) Schott

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Abstract. *Colocasia esculenta* (L.) Schott is an edible aroid from the family of Araceae. It is a tuber crop which is known for its variety of cooking preparation. This study aimed to evaluate the Total Phenolic content (TPC) and antioxidant activities by using 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) scavenging assay. Methanol fraction showed the highest TPC, whereas the extract obtained using 95% ethanol showed the highest inhibition in DPPH scavenging assay and IC_{50} values of 308 µg/mL. These results indicate that *C. esculenta* has antioxidant activity with high phenolic contents which can be consumed as a functional food to increase health benefits and lower the risk of diseases.

1. Introduction

Araceae is the fourth largest monocotyledon plant familes after sedges, grassses and orchids. According to [1] the family comprising of 125 genera with 3, 750 species. The Araceae are widely distributed and mostly were found in trophic Southeast Asia, Malay Archipelago especially Borneo, trophic southern Central America and West Africa [2, 3]. Many reserchers, [4, 5, 6, 7] stated that Araceae is notable importance for the agricultural, food sources and medicinal purposes.

[8] reported the edible Aroids are from the genera of *Colocasia* and *Xanthosoma*, where the most common type of Aroid plant which is edible or ornamental in Malaysia is *Colocasia esculenta* (L.) Schott, also known as taro [9]. They are produced for commercial purposes and are noted by their common names such as *Keladi Sarawak, Keladi Pinang, Keladi Minyak, Keladi Cina,* and *Keladi Mawar*. The plants could be found in moist perennial as well as other wet areas and able to adapt well to changing conditions such as soil types, rainfall, water and temperatures [10]. Besides, [11] stated

that *C. esculenta* is acknowledged for its nutritional benefits as some parts of the plant contain 56.8% of moisture, 1.22% of ash, protein, carbohydrate and starch. It has a high nutritional value which is good to be consumed by people.

The early report by [12] explained that taro has several benefits which are good for human health. It is estimated that 100 gram serving of taro contains about 27% of recommended daily requirement of fibre. In addition, fibre helps in digestive processes as it eases the passage of the digestive system thus resulting in less constipation, bloating, cramping and indigestion.

Besides, taro plant was considered good to human health since the plant has many nutrients content which are minerals, vitamins, lipids and others such as total lipid or fat, energy, carbohydrate, total dietary, protein, sugars, riboflavin, zinc, phosphorus, potassium, manganese and iron. In fact, they also have a higher content of starch than any other tropical root crops available [13]. High source of starch makes the taro plant as staple food for a few countries. However, lack of scientific evidence were available for this crop plant. It is important to know the nutritional values or chemical compound in the taro via extraction for further studies. Therefore, current study was conducted to investigate the total phenolic content (TPC) and antioxidant activity of *C. esculenta* extract prepared in different organic solvents in order to promote its advantages in terms of nutritional values and medicinal potential.

2. Materials and Methods

2.1 Plant Materials and Extraction

The samples of *C. esculenta* were collected *in-situ* from Ayer Lanas, Jeli, Kelantan (Figure 1.A). In this study, the taro's petioles were used as samples instead of corm due to lack of studies regarding petioles and thus we aim to provide the beneficial informations on it. The petioles were cut into pieces and dried in the oven for 3 days at 40 °C (Figure 1.B). After dried, the plant materials were ground using mechanical blender till the samples turn into fine powder (Figure 1.C).

The plant extraction protocol was conducted based on [14], reflux extraction protocol with slightly modifications. A 5 g of powdered samples was added into the round bottom flask with three boiling chips and mixed with 50 mL of the methanol solvent. The solution was heated for 6 h with adjusted temperature according the the boiling point of methanol. The extract obtained were filtered and concentrated using rotary evaporator (Heidolp, Germany). The sample protocol was repeated by using different extarction solvent (ethyl acetate and ethanol).

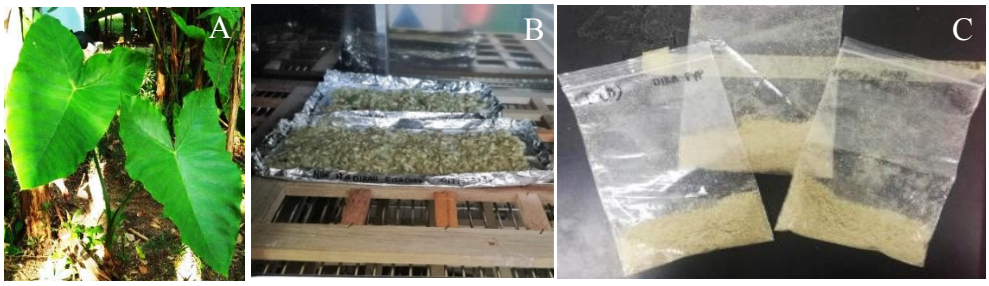

Figure 1. *Colocasia esculenta* (A - *in-situ*, B - corms and petioles, C – powder form).

2.2 Chemicals

2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, Folin ciocalteu's reagent, sodium bicarbonate and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Organic solvents (AR grade)-absolute ethanol, methanol and ethyl acetate 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 of *C. esculenta* extract were determined using Folin-Ciocalteu reagent described by [15] with slightly modifications. Briefly, a 1.0 mg of the crude extract was dissolved in 1 mL of ethanol (1 mg mL^{-1}) and vortex till becomes homogenous stock solution. Various dilutions were prepared from this stock solution. 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 [16] with modifications. Briefly, DPPH solution was prepared by dissolving 0.6 mg of DPPH in 15 mL of ethanol with concentration of 0.1 mM. The working sequence was carried out by pipetting 2.5 mL of the extract with different concentrations (300, 350, 400, 450 and 500 µg/mL) mixed with 2.5 mL of DPPH solution, resulting to total solution equal to 5 mL. After 20 min of incubation in dark, the reading of scavenging effect was measured using microplate reader UV/vis spectromphotometer (FLUOstar® Omega, Germany) at 517 nm. The DPPH scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = [\ (Abs_{control} - Abs_{sample})/\ Abs_{control}] \times 100 \quad (1)$$

Where:

$Abs_{control}$ = Absorbance of DPPH + absolute ethanol

Abs_{sample} = 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.

3. Statistical Analysis

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

4. Results and Discussion

Based on the study conducted, the total extraction yield (%) of *C. longiloba* with methanol, ethyl acetate and ethanol solvent showed 5 g of fine powder has produced approximately 2.4 %, 1.6 % and 3.2% of yields respectively. [17] stated the extraction yield depends on solvents used, duration period and temperature of extraction and the phytochemical content of the plant material itself. Ethanol has high polarity followed by methanol and ethyl acetate. Therefore, the extraction solvent influenced the extraction of *C. esculenta*, where the ethanol solvent was the most suitable to be used as it produced the highest percentage of yields to compare to methanol and ethyl acetate. The extract was used in the following test to obtain scientific data.

4.1 Total phenolic contents (TPC)

Total phenolic contents from the different extraction solvent of *C. esculenta* are shown in Table 4.1.

Table 4.1. The total phenolic content of *C. esculenta* in different solvents.

Solvent	Total phenolic content (mg GAE g^{-1})
Methanol	34.345±0.001
Ethyl acetate	25.885±0.001
Ethanol	30.367±0.006

Values are expressed as mean±standard deviation.

Methanolic extract has the highest value of total phenolic contents, followed by ethanol and ethyl acetate. This indicates the chemical compounds from *C. esculenta* diffused well in methanol solvent, similarly with the finding obtained by [15] – the total phenolic content of methanolic extract was the highest compared to another solvent. Polar solvents are commonly used for recuperating polyphenols from plant tissues [16]. The chemical constituents in plant itself may or may not soluble in a specific solvent, thus the extraction solvent play critical role in extracting the bioactive compounds from plant [18]. According to [19], phenolic components have redox properties as some antioxidants. The phenolics influenced the oxidative stress tolerance on plants which are caused by biotic and abiotic stress situations that produce reactive oxygen species (ROS) in plants. The high content of phenolic influenced the properties of the crude extract. Most of the crude extracts from vegetables, herbs, fruits and other plant materials that rich in phenolic are highly used in the food industry for their antioxidative properties and health benefits.

4.2 Antioxidant activities

In this study, it is showed (Figure 4.2.A) the ethanolic extract of *C. esculenta* exhibit the highest percentage of inhibition in each concentration. At concentration 500 µg/mL, ethanolic extract reached 74.11 % inhibition, whereas for methanolic and ethyl acetate extract, the percentage of inhibition are 57.33 % and 40.78 % respectively.

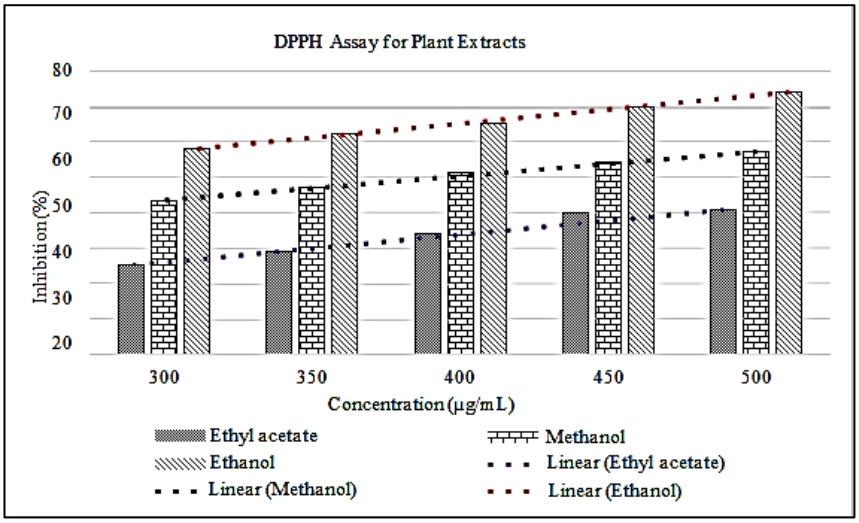

Figure 4.2.A. DPPH Assay comparing the inhibition (%) of the plant extracts different concentrations.

Studies by [16] reported similar result that the extract obtained by ethanol exhibit the highest DPPH radical scavenging activity. Whereas, the findings by [20] revealed *C. esculenta* petioles had the highest antioxidant activity compared to root and leaf. Table 4.2.B showed the mean and standard deviation readings for ethanol was 65.96±6.24 %, while for methanol and ethyl acetate was 50.71±5.57 % and 33.76±6.80 % respectively.

Table 4.2.B. DPHH radical scavenging assay for *C. esculenta*.

Extract	DPPH Radical Scavenging Assay
Ethyl acetate	33.76±6.80 %
Ethanol	65.96±6.24 %
Methanol	50.71±5.57 %

* values are expressed as average ± standard deviation of triplicate measurements.

IC_{50} or half maximal inhibitory concentration of a compound is the amount of antioxidant needed to decrease 50% of the DPPH concentration. It is inversely related to its antioxidant capacity which can be obtained by interpolation from a linear regression analysis. Based on the results from Figure 4.2.C, the concentration for ethanolic plant extract achieved it 50% of inhibition with value 308 µg/mL. Plant extracts with a lower value of IC_{50} indicate that their antioxidant activity is higher [16]. The value of IC_{50} for the plant extracts in which the ethyl acetate recorded the highest IC_{50} value. It can be interpreted that ethyl acetate has lower antioxidant activity as the higher value of IC_{50} represented the lower antioxidant activity. As for methanol, the value was in between ethanol and ethyl acetate thus indicating that the antioxidant activity of those solvents were moderate.

In a study done by [20], the inhibition concentration of the *C.esculenta* stem for IC_{50} was 0.125 ppt (parts per trillion, $10^{\wedge-12}$), as for the leaf extract was 0.28 ppt and the highest value was from the corm or tuber with 4.8 ppt. These studies were done to determine the antioxidant activity as well as the anticancer activity of the species. It was found that at the concentration of 20 and 30 µg/mL, the stem and root of the samples indicated 30% of cancer cell whereas there was no anticancer activity observed in the leaf of the samples.

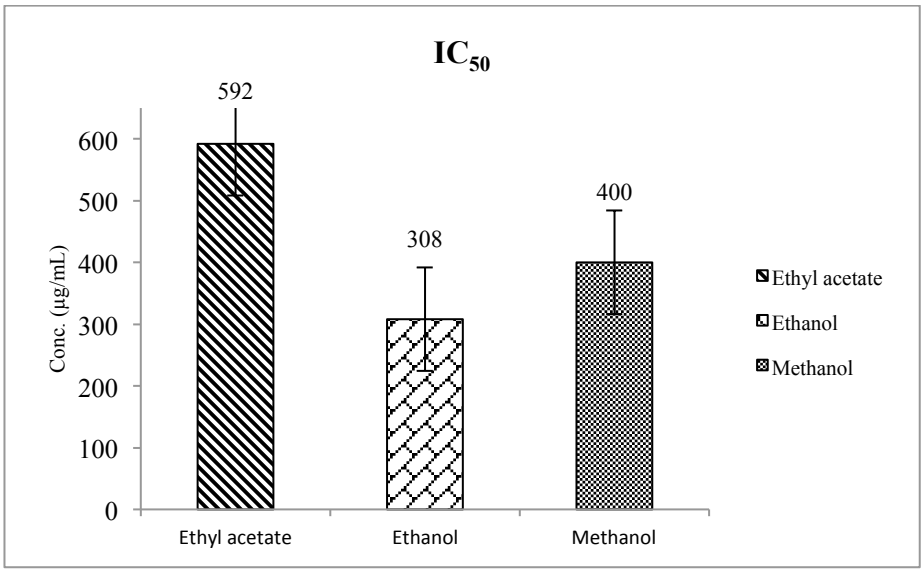

Figure 4.2.C. The value of IC50 for the plant extracts.

5. Conclusions

C. esculenta is an edible tuber crop believed to contain high nutritional values as human diet. From current study, the extract of *C. esculenta* petioles exhibited a fair value of total phenolic content as well as the antioxidant activity. It resulted in higher total phenolic content for methanol solvent compared to ethanol and ethyl acetate. The DPPH scavenging assay showed that ethanolic extracts inhibit higher antioxidant activity. Thus, it is evidenced that the edible *C. esculenta* can potentially be

a natural source of antioxidant due to the presence of bioactive compounds that may promote human health.

Acknowledgments

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