Total phenolic content and antioxidant activity of limestone endemic Araceae species, *Alocasia farisii*

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H M Puteri-Adiba[1], M Arifullah[2], A A Nazahatul[1], T Sirikitputtisak[3], S Klaiklay[4], P Chumkaew[5], S Chewchanwuttiwong[5], M Z Norhazlini[1] and H Zulhazman[1*]

[1]Department of Natural Resources and Sustainability Science, Faculty of Earth Science, Campu Jeli, Universiti Malaysia Kelantan, Locked Bag 100, 17600 Jeli, Kelantan
[2]Faculty of Agro-Based Industry, Campus Jeli Universiti Malaysia Kelantan, Locked Bag 100, 17600 Jeli, Kelantan
[3]International Affairs & Cooperative Education, Prince of Songkla University, Surat Thani Campus, Thailand
[4]Department of Chemistry & Centre of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkla 90112, Thailand
[5]Faculty of Science & Industrial Technology, Prince of Songkla University, Suratthani Campus, Suratthani 84000, Thailand
*Corresponding author: zulhazman@umk.edu.my

Abstract. The changing environments are giving a rise to free radical, causing development of degenerative disease. A search for natural antioxidant is required as the synthetic antioxidant reported has carcinogenic effects on living organisms. Therefore, the aim of this study is to determine the total phenolic content and antioxidant activity of *Alocasia farisii* leaves and petioles using three different polarity solvent which are methanol, ethanol and ethyl acetate. The total phenolic content was evaluated using the Folin-Cioucalteu reagent with some modification and the antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay. The methanolic extract attained the highest total phenolic content and antioxidant activity at 46.615 µg GAE/g and 66.43 %, respectively. Ethyl acetate with the lowest polarity had the lowest value, 34.769 µg GAE/g total phenolic content and 58.274 % in antioxidant activity. The IC_{50} value shows methanol recorded the lowest value at 339.905 µg/mL, indicates high radical scavenging activity whereas ethyl acetate has highest IC_{50} value (400 µg/mL) indicates low radical scavenging. These finding provide useful information on the total phenolic content and antioxidant activity of *A. farisii* that can be a reference for further research on this species of Araceae family. The leaves and petiole extracts of *A. farisii* may be exploited as natural sources of antioxidant.

1. Introduction

The changes in environmental conditions are giving rise to a variety of free radicals causing development of degenerative diseases [1]. Plants are the main natural resources that human needs to support their life because it contains variety of secondary products useful as natural remedy, able to scavenge the free radicals [2]. [3] noted that synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertbutylhydroquinone (TBHQ) are responsible for carcinogenesis and liver damage in living organism. Therefore, the searching of natural antioxidant

agents has attracted much interest plus many of the plants are known to contain large amount of phenolic that act as antioxidant [4, 5, 6].

Araceae is the fourth largest family of monocotyledons after orchids, grasses and sedges. The Araceae family comprises of 125 genera and 3,750 reported species widely distributed in the humid tropics [7, 8, 9, 10]. Studies on Araceae had been conducted since 15th century [11]. The uses of *Alocasia* as: i) food; *Alocasia fornicata* and *A. macrorhiza* are an important food source in Asia and Africa [12, 13]. Almost all parts of these plants are used as food due to their richness in starch and the antioxidant properties of its edible parts have also been established. The plants were widely used as ii) ornamental foliage due to their beautiful and unusually diverse leaf forms and textures [14, 15]. According to [16], Araceae commonly used as iii) traditional medicines by ancient cultures to treat wounds, insect bites, and healing of stings [17] also noted that *Alocasia* species have a potential use medicinally.

The *Alocasia* was mostly studied for antioxidant, antitumor and cytotoxic studies which are mostly related to cancer studies [17]. The presence of antioxidants as phenolics, flavonoids, proanthocyanidins and tannins found in fruits, flower, leaves and petioles may provide protection against various numbers of diseases [18, 19, 20, 21]. *Alocasia farisii*, a remarkable new described species of Araceae found from Karst limestone area in Kelantan, Peninsular Malaysia. The species is almost similar to the Bornean *Alocasia reversa* except the staminate flower zone that is only half enclosed in the lower spathe chamber [22]. However, since *A. farisii* is a new species, the total phenolic compound and antioxidant activity has not been explored yet. Therefore, this study was aimed to determine the total phenolic content and antioxidant activity of *A. farisii*.

2. Materials and methods

2.1. Plant materials and extraction

The samples of *A. farisii* were collected from limestone area at Gua Ikan, Kelantan (05º21’14.5’’N 102º01’44.5’’E). In this study, the *A. farisii* leaves and petioles were used as plant materials. The samples were cut into pieces and dried in the oven at 40 ℃ for a week. After dried, the samples were grind using mechanical blender till the samples turn into fine powder. Then, the powder was sieved and divided into three portions, weighted before extracted through reflux extraction protocol by using methanol, ethanol and ethyl acetate. The extracts obtained were filtered using Whatmann’s No. 1 filter paper and concentrated at 42 ℃ using rotary evaporator (Buchi R-100). All the extracts were stored in cold storage at 4 ℃ for further analysis.

2.2. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu’s reagent, sodium carbonate 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 *A. farisii* were conducted by using Folin-Ciocalteu reagent [23]. Briefly, 200 µL of the extracts (1 mg mL^{-1}) were made up to 3 mL with deionized water, mixed thoroughly with 0.5 mL of Folin-Ciocalteu reagent. After 5 min of incubation, 2 mL of 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 650 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 µg gallic acid equivalents (GAE) per gram of sample (µg/g). All tests were conducted in triplicates.

2.4. Determination of antioxidant activity (DPPH scavenging assay)

The DPPH assay was carried out based on method described by [24] with modifications. Briefly, DPPH solution was prepared by dissolving 0.6 mg of DPPH in 40 mL of ethanol with concentration of 0.01 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 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 tests were carried out in triplicates. The data were analysed using the Statistical Package for the Social Sciences (SPSS) and were presented in mean±standard deviation.

4. Results and discussion

Based on the study conducted, the total extraction yield (%) of *A. farisii* with methanol, ethanol and ethyl acetate solvent had produced approximately 18.4 %, 16.81 % and 5.62 % of yields respectively. This suggests that the major phytochemicals in *A. farisii* are mostly high in polarity and soluble in methanol because the extraction yield increases with increasing polarity of the solvent used in the extraction. The result was supported by the findings reported by [25] on *Helicteres hirsute* and [26] on *Paramignya trimera*, where methanol solvent produced the highest extraction yield whereas ethyl acetate produced the lowest yield.

4.1. Total phenolic content (TPC)

Total phenolic contents from the different extraction solvents of *A. farisii* are shown in Table 1. Methanolic extract has the highest content of phenolic compounds followed by ethanol and ethyl acetate extract with the value 48.615±0.005, 43.359±0.007 and 34.769±0.004 µg GAE g^{-1} respectively.

Table 1. Total phenolic content of *A. farisii* in different organic solvent.

Solvent	Total phenolic content (µg GAE g^{-1})
Methanol	48.615±0.005
Ethanol	43.359±0.007
Ethyl acetate	34.769±0.004

*Values are expressed as mean ± standard deviation.

This indicates methanol as the polar solvent able to extract the phenolic compounds in *A. farisii* efficiently. According to [27], the higher polarity solvent has better solubility for phenolic compounds present in plant samples. Similar findings reported by [28] on *Myrtus communis* leaves and berries where the total phenolic content as following orders: methanol > ethanol > ethyl acetate.

4.2. Antioxidant activity (DPPH radical scavenging assay)

From the studied, the data obtained presented in Table 2 showed the antioxidant activity of *A. farisii* is directly proportional to the concentration of plant extracts. As the concentration of plant extract increases (300 to 500 µg mL^{-1}), the antioxidant activity also increases. The ranges of radical scavenging activities from three different extracts were between 45.272 ± 0.001 to 66.43 ± 0.004 %. At concentration of the extract 500 µg mL^{-1}, the highest antioxidant activity was obtained with the methanol (66.43 %), followed by ethanol (64.894 %) and ethyl acetate (58.274 %). Similar findings were reported by [29] on *Marrubium peregrinum* L. that methanol extract has higher value of antioxidant activity than ethyl acetate.

Table 2. The antioxidant activity (DPPH radical scavenging assay) of *A. farisii*.

	*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	51.891±0.003	55.083±0.002	61.466±0.001	63.83±0.004	66.43±0.004	339.905
Ethanol	51.182±0.002	54.374±0.006	58.156±0.002	60.165±0.001	64.894±0.006	352.113
Ethyl acetate	45.272±0.001	47.281±0.001	51.064±0.004	52.01±0.002	58.274±0.007	400

*values were expressed as average ± standard deviation.

The IC_{50} value is important to know the amount of plant extract needed to decrease the absorbance of DPPH by half-maximal (50%) [30]. Based on Table 2, it can be seen that methanol has the lowest IC_{50} value, which indicates high antioxidant activity as it able to neutralize 50 % of free radicals at the concentration 339.905 µg mL^{-1}. A moderate activity was found in ethanol with IC_{50} value of 352.113 µg mL^{-1} and ethyl acetate recorded the highest value, 400 µg mL^{-1}, indicating low antioxidant activity. [31] reported similar finding on *Juglans regia* L., where the IC_{50} value as following order: methanol < ethanol < ethyl acetate. Besides, Mandal et al. (2010) study on *A. fornicata* also shows IC_{50} value at 128.07 µg mL^{-1} in ethyl acetate extract which is lower than the values obtained in the present study.

5. Conclusions

In conclusion, the objectives of this study were achieved as it provides the data and information regarding the total phenolic content and antioxidant activity of *A. farisii* leaves and petioles. The polar solvents (methanol) exhibited the highest value for both of the test, compared to lowest polar solvent (ethyl acetate). This highlights that most phytochemicals in *A. farisii* are soluble in polar solvent. The results of this study provide useful information on the total phenolic content and antioxidant activity of *A. farisii*, that can be reference for further research on this species of Araceae family. Besides that, the leaves and petioles of *A. farisii* may be exploited as sources of natural antioxidant.

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