DETERMINING THE TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF AROID (*ALOCASIA FARISII*)

by:

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A report submitted in fulfilment of the requirement for the degree of Bachelor of Applied Science (Natural Resources Science) with Honours.

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APPROVAL

"I hereby declare that I have read this thesis and in my opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Bachelor of Applied Science (Natural Resources Science) with Honours"

Signature	:	…….....
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Date	:	……..

DECLARATION

I declare that this report entitled "Determining the Total Phenolic Content and Antioxidant Activity of Aroid (*Alocasia farisii*)" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ……

Name : Puteri Adiba Hasanah Binti Mohamed Idrus

Date : ……

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Determining the Total Phenolic Content and Antioxidant Activity of Aroid *(Alocasia farisii)*

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ABSTRACT

Problem statement: The changing environment are giving a rise to free radical causing development of degenerative disease. **Significant of study**: A search for natural antioxidant is required as the synthetic antioxidant reportedly has carcinogenic effect on living organisms. **Objectives:** The aim of this study is to determine the total phenolic content and antioxidant activity of *Alocasia farisii* leaves and petioles using three different solvent polarity which are methanol, ethanol, ethyl acetate. **Method:** 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) method. **Results:** The methanol extract, high polarity solvent attained the highest total phenolic content and antioxidant activity at 48.615 µg GAE/g and 6.43%, respectively. Ethyl acetate, a low polarity solvent recorded the lowest content compared to others at 34.769 µg GAE/g in total phenolic content and 45.272% in antioxidant activity. The IC50 value was obtained by plotting the scavenging activity (%) against concentration of plant extracts. Methanol recorded the lowest at 339.905 µg/mL, which means it has a high radical scavenging activity and ethyl acetate at the highest at 400 µg/mL, a low radical scavenging activity. The correlation of total phenolic content and IC50 showed that total phenolic content was negatively correlated with IC50 of DPPH. **Conclusion:** These finding provide useful information on the total phenolic content and antioxidant activity of *Alocasia farisii* that can be a reference for further research on this species of *Araceae* family. The leaves and petiole extracts of *Alocasia farisii* may be exploited as sources of natural antioxidant

Keywords: Total phenolic compound; antioxidant activity; aroid; *Araceae* family; *Alocasia farisii*

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Menentukan Jumlah Kandungan Fenolik dan Aktiviti Antioksidan Keladi (*Alocasia farisii*)

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ABSTRAK

Pernyataan masalah: Persekitaran yang berubah-ubah memberikan peningkatan radikal bebas yang menyebabkan perkembangan penyakit degeneratif. **Kepentingan pembelajaran**: Oleh itu, pencarian antioksida semulajadi diperlukan kerana antioksida sintetik direkodkan memberi kesan karsinogenik kepada organisma hidup. **Objektif**: Tujuan kajian ini adalah untuk menentukan jumlah kandungan fenolik dan aktiviti antioksidan dalam daun dan batang *Alocasia farisii* menggunakan tiga pelarut yang berbeza iaitu metanol, etanol, etil asetat. **Kaedah**: Jumlah kandungan fenolik telah dinilai menggunakan kaedah reagen Folin-Cioucalteu dengan beberapa pengubahsuaian dan aktiviti antioksidan oleh kaedah 1,1-diphenyl-2-picrylhydrazyl (DPPH). **Keputusan**: Ekstrak metanol, polar tinggi dicatatkan sebagai yang tertinggi dalam kandungan total fenolik dan aktiviti antioksidan pada 48.615 µg GAE / g dan 66.43%. Manakala ekstrak etil asetat, pelarut polariti rendah mencatatkan jumlah kandungan total fenolik dan aktiviti antioksida paling rendah berbanding dengan yang lain pada 34.769 µg GAE / g dan 45.272%. Nilai IC_{50}diperolehi dengan merancang aktiviti pemotongan (%) terhadap kepekatan ekstrak tumbuhan. Methanol mencatatkan paling rendah pada 339.905 µg / mL manakala etil asetat paling tinggi pada 400 µg / mL. Hubungan antara jumlah kandungan fenolik dan IC_{50} menunjukkan bahawa jumlah kandungan fenolik adalah negatif berkorelasi dengan jumlah IC_{50} DPPH. **Kesimpulan**: Pembelajaran ini menunjukkan jumlah kandungan fenolik and antioksidan aktiviti daun dan batang *Alocasia farisii* yang boleh dijadikan sebagai bahan rujukan untuk pembelajaran selanjutnya. Ia juga terbukti berpotensi dijadikan sebagai sumber antioksidan asli.

Kata kunci: Jumlah kandungan fenolik; aktiviti antioksidan; keladi; keluarga *Araceae*; *Alocasia farisii*

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LIST OF ABBREVIATIONS

(w/v)	Weight per volume
H_2O_2	Hydrogen Peroxide
O_2	Oxygen
O_2^-	Superoxide anion
µg/mL	microgram per millilitre
µL	microliter
1,1-diphenyl-2-picryl-hydrazy	DPPH
℃	Celsius
g	gram
mg/mL	milligram per millilitre
mL	millilitre
mm	millimolar
mM	millimolar
nm	nanometer
UMK	University Malaysia Kelantan
OH	Hydroxyl Radical

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Studies on aroid has been conducted since 15th century (Croat, 1998). Aroid or *Araceae* is the fourth largest monocot families after orchids, grasses and sedges (Yuzammi, 2018). It is also one of the most important mesophytic plant families in tropical Asia (Mansor, Boyce, Othman, & Sulaiman, 2012). The *Araceae* family comprice of 125 genera and about 3,750 species, most of them widely distributed in the humid tropics (Boyce & Croat, 2011)

Aroid for the greater part are plants that depend on abundant available water and prevailing high atmospheric humidity. Therefore, most of them are widely distributed in the humid tropics like tropical America, mainland Southeast Asia and Malesian region (Boyce & Croat, 2011). The distinctive features of all aroids are the beauty with some bizarre combination of spadix and spathe called the inflorescences which referred to as a flower. (Boyce & Wong, 2015).

Over the past few decades, the change in environmental conditions are giving rise to a variety of free radicals causing development of degenerative diseases (Mulla, Salunkhe, Kuchekar, & Qureshi, 2009). Free radicals involved in several pathological conditions such as liver disorders, diabetes atherosclerosis and nephrotoxicity as it inactivate enzymes and damage important cellular components. (Arora, Sairam, &

Srivastava, 2002). The search of natural antioxidant agents have attracted much interest due to their ability to scavenge these free radicals (Li, Wang, Li, Li, & Wang, 2008).

According to Bown (2000), aroids have been used as herb medicines in many ancient cultures for healing of stings, insect bites, wounds and skin complaints. Results also show that *Alocasia* species are mostly studied for antioxidant, antitumor and cytotoxic studies which are mostly related to cancer studies (Ongpoy, 2017). The presence of antioxidants such as phenolics, flavonoids, proanthocyanidins and tannis found in fruits, flower, leaves and petioles of plants may provide protection against various number of diseases (Baba & Malik, 2015). According to Yen, Duh, & Tsai (2002), many of these parts are known to contain large amounts of phenolic antioxidants

Although *Alocasia* species has potential use for medicinal value, few studies have been conducted on the pharmacological activities of the plant. Phenolic compounds are common in the kingdom of plant as they scavenge free radicals and act as antioxidants. However, since *Alocasia farisii* is a new species, the total phenolic compound and antioxidant activity has not been determined. Therefore, the objective of this study is to determine the total phenolic content and antioxidant activity of aroid (*Alocasia farisii*).

1.2 Problem Statement

The change in environmental conditions are giving rise to a variety of free radicals causing development of degenerative diseases (Mulla et al., 2009). Plants are the main natural resources that human needs to support their life because it contains of variety

of biochemical products, many of which are extractable and found useful in a number of pharmaceutical preparations. Amiri (2010), noted that synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) can be responsible for carcinogenesis and liver damage in living organism. Therefore, the search of natural antioxidant agents have attracted much interest due to their ability to scavenge free radicals (Li et al., 2008).

The presence of antioxidants such as phenolics, tannins and flavonoids in the plants may provide protection against a number of diseases (Baba & Malik, 2015). According to Yen et al. (2002), many of the plants are known to contain a large amount of total phenolic content that act as antioxidant. Ongpoy (2017) also noted that *Alocasia* species have a potential use medicinally. However, since *Alocasia farisii* is a new species, the total phenolic content and antioxidant activity is not known.

1.3 Objective of Study

This study will mainly focus on the following objectives:

1) To determine the total phenolic content in *Alocasia farisii*
2) To study the antioxidant activity in *Alocasia farisii.*

1.4 Scope of Study

In this research, the area of study is limited to the total phenolic content and antioxidant activity of *Alocasia farisii*. Ten sample of *Alocasia farisii* were obtained from the area of limestone at Gua Ikan, Kelantan. The plant leaves and petioles are the

parts that were used for the extraction process using three different solvents which are methanol, ethanol and ethyl acetate.

The method used to test the total phenolic content and antioxidant activity are Folin-Ciocalteu and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. The results were then used to analyse the total phenolic content and antioxidant activity in *Alocasia farisii* that can contribute to as a potential medicinal value for treatment of many diseases.

However, this study has some limitation such as there are not many literatures that can be review as *Alocasia farisii* is a new species. It should be noted that, the total phenolic content and antioxidant activity is not applicable to the whole plant as only leaves and petioles were used.

1.5 Significant of Study

Nowadays, there are growing industrial interests on the production of herbal antioxidants as it can be supplemented in food, medicine and cosmetics (Mandal, Misra, & Singh, 2010a). This is because the synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and *tert*-butylhydroquinone (TBHQ) can invoke carcinogenesis and liver damage in living organisms (Amiri, 2010). The presence of antioxidants such as phenolics, flavonoids and tannins in the plants may provide protection against a number of diseases (Baba & Malik, 2015).

Results showed that *Alocasia* species have a potential use for medicinal cases (Ongpoy, 2017). However, there are less knowledge on value of *Alocasia farisii* because it is a new species. Therefore, this research is concerned to analyse for total phenolic content and antioxidant activity in *Alocasia farisii* leaves and petioles. The

result of total phenolic content can be used by any related party as reference for further researches on *Alocasia farisii*. Meanwhile, the result of antioxidant activity can show the potential of *Alocasia farisii* as natural antioxidant.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to *Araceae*

Aroid or *Araceae* are the fourth largest family of monocotyledons after orchids, grasses and sedges. It contains 125 genera and about 3,750 species, most of them widely distributed in the humid tropics (Boyce & Croat, 2011). Table 2.1 shows that *Araceae* is a member of the Arales or Spathiflrae order (Plowman, 1969) which majority are native to the tropics. It is one of the larger plant families in Malaysia, currently covering 42 described genera and an estimated 12,000 species with the percentage of species either only recently named, or yet to be formally described (Boyce & Wong, 2015). Epiphytes and climbers account for approximately 70% of the total species (Grayum, 1990).

Table 2.1: Taxanomy of *Araceae* family (United States Department of Agriculture, n.d.)

Kingdom	Plantae
Subkingdom	Tracheobionta (Vascular plant)
Superdivision	Spermatophyta (Seed plant)
Division	Magnoliophyta (Monocotyledons)
Class	Liliopsida
Order	Arales
Family	*Araceae*

Based on taxonomic studies (Mayo, Bogner, & Boyce, 1997) and the fossil record from the early Cretaceous period (Cusimano et al., 2011), *Araceae* were discovered to belong to an ancient family with seven subfamilies. Usually, the herbaceous species of *Araceae* has rhizome or tuber with roots that are always dimorphic and adventitious (Mayo et al., 1997).

Inflorescence of *Araceae* are almost always insect pollinated by trigonid bees, flies, beetles, possibly thrips and very doubtfully mites (Mayo et al., 1997). In 1986, Okada results indicated that some species of drosophilid flies are known to breed on the inflorescences of *Alocasia*, *Homalomena* and *Colocasia*. Evidently, odour is the prime factor in attracting pollinators as *Araceae* is famous for its foul inflorescence odour that can be compared to rotten fish, dog faeces and sulphurous gas.

2.2 *Alocasia* Genus

Alocasia is one of the largest and most morphologically diverse genus consisting about 100 species, mainly rhizomatous, sometimes tuber-bearing perennials and were found distributed in subtropical eastern Himalayas, sub-tropical and tropical parts of South and Southeast Asia (Thao et al., 2003). In Peninsular Malaysia, there are only five species, one of which is doubtfully considered native and the other four are invasive (Hay, 1998).

Morphologically, some part of the plant contain an acrid watery or milky juice (Plowman, 1969). The root consists of an elongated or tuberous rhizome from which the aerial parts arise. The leaves are solitary, or few shaped like an expanded blade connected to a petiole. Aroids is an inflorescence and not a flower because it consists of a spike of several or many individual flowers. The floral structure consists of a

flower-bearing protuberance, the spathe and a petal-like leaf, the spathe. The flowers may be perfect or monoecious, with or without a perianth. When flowers are unisexual, the staminate occur on the upper part of the spadix, the pistillate below. During the sterile state, almost all *Alocasia* species have visible waxy glands on the surface of the leaf (Boyce and Wong, 2015). The fruit is a berry with one to many seeds (Mayo et al., 1997).

2.2.1 Uses of *Alocasia* genus

a. Food

Bioactive compounds play a pivotal role for food supply diet. In Asia and Africa, *Alocasia fornicata* (Roxb.) Schott and *Alocasia macrorhiza* (Linn.) G. Don are an important food (Mandal, Misra, & Singh, 2010b). 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. Since nineteenth century, *Alocasia* genus were also used as food for animals. *Alocasia macrorrhizos*, the giant taro, was widely used for animal fodder because the petioles have relatively little mucilaginous irritant component. (Mayo et al., 1997).

b. Ornamental

Ornamental aroids are account for about one-third of total ornamental foliage cultivated and sold for display (Henny et al., 2004). *Alocasia* species are widely cultivated as ornamental plant (Hay, 1998). Their beautiful and unusually diverse leaf forms and textures form an essential part of any tropical plant display especially their

structure of veins. Tropical foliage also proved to be the most successful indoor plants because of their ability to grow under relatively low light and humidity (Ingels, 2010).

c. Medicine

Although *Alocasia* species are widely cultivated as ornamental, their phytomedicinal value also need to be appreciated. This because it contains bioactive compounds, being secondary metabolites having pharmacological or toxicological effects in humans and animals (Chandrasekara & Josheph Kumar, 2016). Secondary metabolites are compound produced in another metabolites, is essential to the functioning of plant after primary metabolites.

In Vietnamese traditional medicine, some species discovered to have curative values. Research shows that local people use *Alocasia macrorhizos* to treat flu, gout, and beriberi (Dan,2011). *Alocasia macrorhizos* was soaked in wine or boil with water and then drunk daily as cure for various diseases. In India, *Alocasia indica* also have been used traditionally used in the treatment of abdomen and spleen related disorders (Pal, Bhattacharjee, Mukherjee, Bhattacharya, & Khowala, n.d.).

2.3 Description *of Alocasia farisii*

Alocasia farisii is a new species of *Araceae* found from Karst limestone area in Kelantan, Peninsular Malaysia as the plant revealed to be undescribed species morphologically congruent with the till now wholly Bornean *Alocasia* princeps complex (Zulhazman et al., 2017). It is almost similar to the Bornean *Alocasia reversa* but dissimilar by having staminate flower zone only half enclosed in the lower spathe chamber.

Alocasia farisii is a small epilithic plant in soil and humus pockets on limestone outcrop and boulders with 55 cm height but mostly about half of this height. The rhizome is elongated with 2.5 cm in diameter. The petiole is in 10 – 25 cm long with pale green colour. Figure 2.1 reveals that the leaf blade is thinly leathery in grey-green colour adaxially with distinctly dark green about the primary veins. It inflorescences together with spathe are usually 7-12 cm long and spadix is shorter than spathe with fruiting spathe berries coloured bright orange in 4 cm long.

Figure 2.1: *Alocasia farisii* at its natural habitat. Adapted from Wiley Online Library by Zulhazman et al., 2017. *Nordic Journal of Botany 35*, p. 301. Copyright 2017 by the Nordic Society Oikos.

2.3.1 Distribution and Ecology

According to Zulhazman et al. (2017), *Alocasia farisii* distribution so far are known to be from the Karst limestone areas of Jentian (Tanah Merah, ca 05°29′14.5″N, 102°01′44.5″E), Gua Ikan–Gua Pagar (Kuala Krai, ca 05°21′14.5″N, 102°01′35.3″E), and Gua Musang, Kelantan, northeast Peninsular Malaysia. *Alocasia farisii* is a lithophytes plants that grows in soils and humus pockets of limestone. However, it also rather occasionally grows as terrestrial on forested Karst limestone.

2.4 Formation of Free radical

Free radicals and other active derivatives of oxygen are inevitable by-products of biological redox reactions. The increased production of toxic oxygen derivatives is a common feature of stress conditions as shown in figure 2.2. For example, about 5% or more of the inhaled O_2 is converted to reactive oxygen species (ROS) such as O_2^-, H_2O_2 and OH by univalent reduction of O_2 (Gupta & Sharma, 2006). Free radical made by peroxidation cause development of degenerative diseases (Cross et al., 1987). Mulla et al. (2009), note that free radicals inactivate enzymes and damage important cellular components by causing tissue injury through lipid peroxidation and covalent binding. Food quality deterioration is one of the cause of lipid peroxidation (Babbar, Oberoi, Sandhu, & Bhargav, 2014). It also involved in several pathological conditions such as liver disorders, atherosclerosis, diabetes and nephrotoxicity.

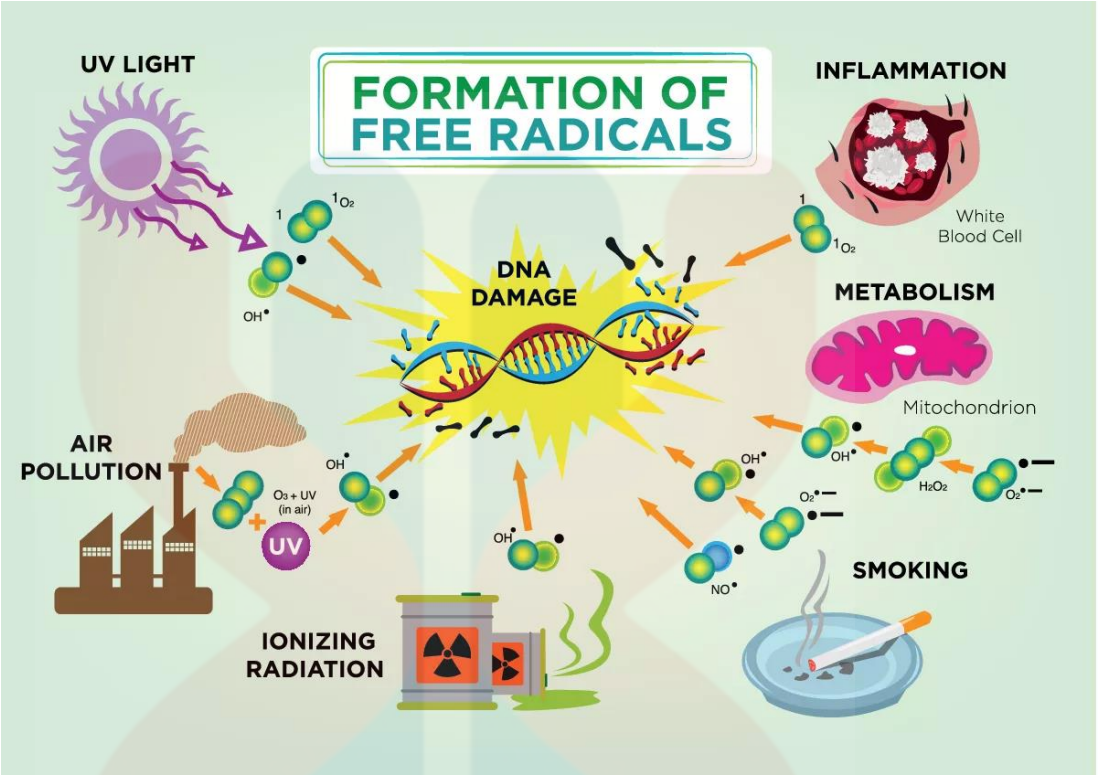

Figure 2.2: Formation of free radicals. Reprinted from "What are free radicals, anyway? -*Solesence*," n.d.. Retrieved December 12, 2018, from https://solesence.com/what-are-free-radicals-anyway/. Copyright © 2018 Solesence.

In order to contend the rise of free radical due oxidative stress, antioxidant is needed. An antioxidant is any substances that, when present at low concentration can significantly delays or prevent free radicals of cell content (Hallowes & Lewis, 1971). Catalase, dismutase, superoxide dismutase, and glutathione peroxidase are the examples of natural antioxidants present in our body, however they are not enough for severe or continued oxidative stress (Li et al., 2008). While there are synthetic antioxidant like butylated hydroxyl toluene and butylated hydroxyl anisole, they are suspected to be carcinogenic and hence no longer in use (Amiri, 2010).

Plant and other organisms have evolved a wide range of mechanisms to cope with this problem. The antioxidant defence system of the plant comprises a variety of

antioxidant molecules and enzymes. Therefore, in recent years the search of antioxidant from natural origin has been greatly felt (Jayaprakasha, Singh, & Sakariah, 2001). Antioxidant may resist the oxidative stress by quenching the free radicals, inhibiting the free radicals, inhibiting the lipid peroxidation and can prevent diseases (Mulla et al., 2009).

2.5 Phenolic Compound

Phenolic compounds are known to be broadly distributed in plants. Phenolic compound is a secondary metabolites which derivatives of the growth and development in plants (Randhir, Lin, & Shetty, 2004). These compounds one of the most commonly occurring groups of phytochemicals, significantly physiological and morphological important in plants. Scavenge free radical activity had been reported in phenolic compounds. It found in both nonedible and edible plants and several biological effects as well as antioxidant activity. According to Amensour, Sendra, Abrini, Pérez-Alvarez, & Fernández-López (2010), redox properties of phenolic have been recorded to be attributable as antioxidant activity.

As one of the most important antioxidant plant components, phenolic compounds have been widely investigated in many medicinal plants, fruits, and vegetables (Djeridane et al., 2006). The redox properties of phenolic antioxidant play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Amensour et al., 2010). Epidemiological and *in vitro* studies positively suggest that foods containing phytochemicals with anti-oxidation potential have high protective effects against major disease risks such as cancer and cardiovascular diseases (Kaur & Kapoor, 2002).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Location

For this study, ten samples of *Alocasia farisii* plant were collected from limestone area at Gua Ikan, Kelantan (Appendix A). It is a lithophytes plants that grows in soils and humus pockets of limestone. The plant was dried and turn into powder at B.A.P 1.1 laboratory in Universiti Malaysia Kelantan. Meanwhile, the extraction and analysis process were conducted three months after that in Prince of Songkla University, Surat thani Campus.

3.2 Material

Glassware items such as beakers, volumetric flasks, measuring cylinder, test tube, centrifuge tube, round bottom flasks, condenser, boiling chip, glass rod and cuvette were rinsed with distilled water for sterilization. Retort stand, spatula, test tube tray, Whatmann no 1 filter paper, micropipette (10 ÷ 1000 µL) and blender were used when carrying out this study. Ovens, weighing machine, magnetic stirrer hot plate, rotary evaporator, UV visible spectrophotometer are some of the laboratory machine that were used in this study.

Chemicals that are used in this study is methanol, ethyl acetate, ethanol, deionized and distilled water, gallic acid, Folin-Ciocalteu reagent, sodium carbonate, 1,1-diphenyl-2-picryl-hydrazy (DPPH) and ascorbic acid.

3.3 Method

3.3.1 Preparation of Plant Extract

The *Alocasia farisii* leaves and petioles were rinsed several times through running tap water then followed with distilled water. Each parts of the plant were cut into small pieces and dried in the oven at 40℃ for one week. Blender were then used to reduce the particle size of sample until it became a powder. The powder was then sieved and divided into three portions to be weighted before extracted through Reflux technique with methanol, ethanol and ethyl acetate. Each crude extract was later filtered using Whatmann's No.1 filter paper. The filtrates extracts were then concentrated at 42 °C using a rotary evaporator (Buchi R-100). All the plant extracts were dried, weighted and stored in a refrigerator at 4℃ for further analysis.

3.3.2 Total Phenolic Content Assay

The total phenolic content (TPC) of each extract were carried out by using the method of Folin-Ciocalteu (Kaur & Kapoor, 2002). An aliquot 200 µL of extract (1 mg/mL) or standard solution of gallic acid (20, 40, 60, 80, and 100 µg/mL) were made up to 3 mL with deionized water, mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for 5 minutes and 2 mL of 20% (w/v) sodium carbonate were added. A blank reagent using ethanol were prepared. The mixture was then allowed to stand in the dark at room temperature for a further 60 minutes, and absorbance of mixture were measured against blank reagent at 650 nm using UV-visible spectrophotometer. TPC was stated as µg gallic acid equivalents (GAE).

$$TPC = C \times \frac{V}{M} \quad (3.1)$$

where, C = concentration of gallic acid established from the calibration curve (µg/mL)

V = volume of the extract solution (mL)

M =weight of the extract (g)

3.3.3 Antioxidant properties Assay

The antioxidant activity of the extract were determined by using the DPPH method (Do et al., 2014) with some modification. The DPPH solution were freshly prepared by dissolving 0.0006 g of DPPH in 40 mL of ethanol (about 0.01mM). An aliquot 2.5 mL of each extract with varying concentration (300,350,400,450 and 500 µg/mL) were mixed with 2.5 mL DPPH solution and incubated in the dark at room temperature for 20 minutes. The absorbance of the mixture against reagent blank was measured at 517 nm using UV-visible spectrophotometer. Ascorbic acid was used as a positive control. The ability of the sample to scavenge DPPH radical was determined from:

$$\% \text{ inhibition} = \frac{Control_A - Sample_A}{Control_A} \times 100 \quad (3.2)$$

where, A = absorbance measurement (nm)

3.4 Statistical analysis

All the experiments were conducted in triplicate measurement. The mean and standard deviation were calculated using Microsoft Office Excel Software (Babbar et al., 2014). The total phenolic content was calculated using equation 3.1. Next, the percentage (%) inhibition was calculated using equation 3.2 whilst IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm. Correlation regression using Microsoft Office Excel Software were used to show the relationship between total phenolic content and IC_{50}.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Percentage Yield of Extracts

The leaves and petioles of *Alocasia farisii* were cut into smaller pieces and dried in the oven as a method of preservation. The dried leaves and petiole were then blended into powder. Lowering the particle size increases surface contact between samples and extraction solvents (Azwanida, 2015). Powdered samples underwent the Reflux technique using three different solvents to extract out polar compounds which constitutes the bulk compounds present in the samples.

The solvents used in this study were selected based on their different polarity ranges. According to Zuo, Chen, & Deng (2002), non-polar substance will dissolve in non-polar solvent while polar substances would dissolve in polar solvents. Methanol, ethanol and ethyl acetate (in order of decreasing polarity, respectively) were used to enable the extraction and separation of a wide range of components that are present in the samples.

The percentage yield for the various solvent extract of leaves and petioles of *Alocasia farisii* is shown in table 4.1. For each of plant extract, the yield was expressed in percentage by dividing the quantity of dry mass obtained after extraction by the dry weight of the powder used before soaking.

Table 4.1: Percentage yield of various solvent extract of leaf and petiole of *Alocasia farisii*

Extract	Weight of powder (g)	Mass of extract (g)	% Yield
Methanol	1.25	0.23	0.18
Ethanol	1.13	0.19	0.17
Ethyl Acetate	2.49	0.14	0.06

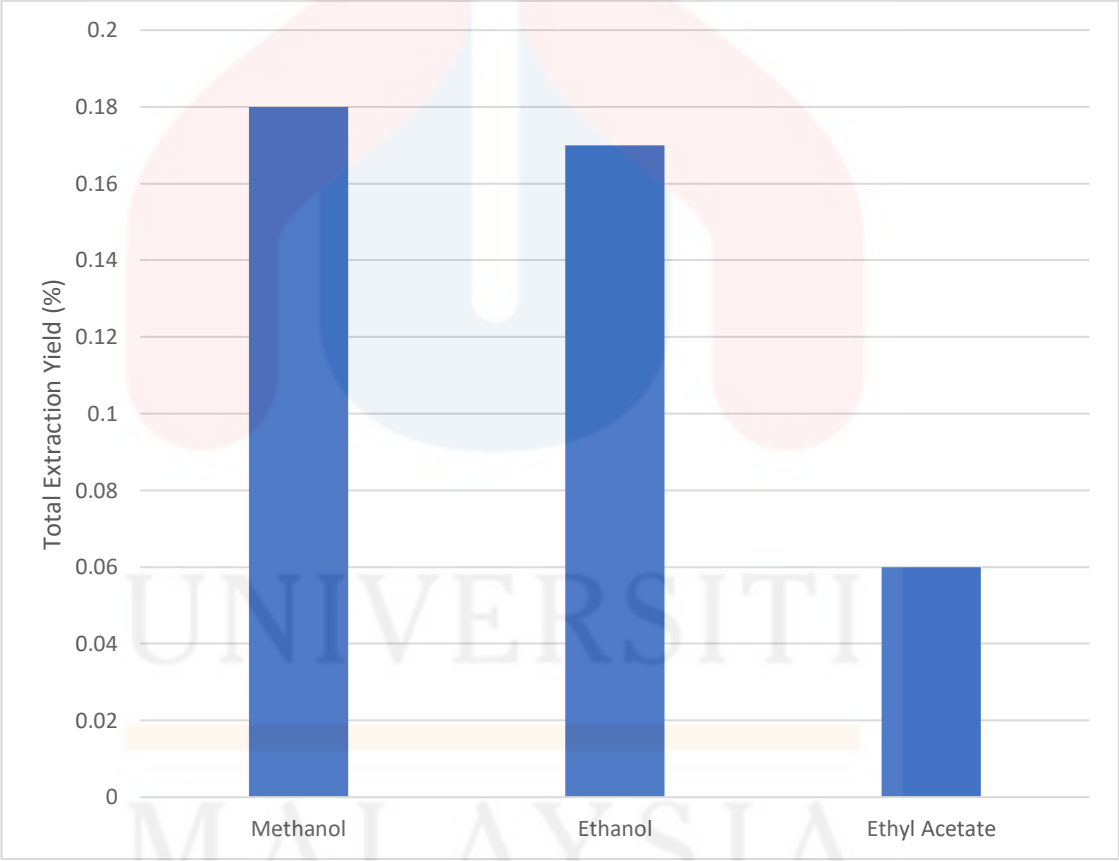

Figure 4.1: The total extraction yield of *Alocasia farisii* plant material by Reflux extraction according to respective solvent.

Figure 4.1 shows that methanol solvent has the highest extraction yield with 0.18% while ethyl acetate has the lowest with 0.06% extraction yield. Although the weight of plant powder was the highest in ethyl acetate, it dropped to the lowest extraction yield. This suggests that the major phytochemicals in *Alocasia farisii* are mostly high in polarity and soluble in methanol because the extraction yield increases with increasing polarity of the solvent used in extraction. The result of the present study resembled on the studies conducted on *Helicteres hirsute* Lour. leaves (Ngoc et al., 2015) and *Paramignya trimera* (Nguyen, Bowyer, Vuong, Altena, & Scarlett, 2015) where methanol is the highest extraction yield and ethyl acetate with the lowest extraction yield. Thus, it shows that there are plants having same property with *Alocasia farisii*, where the bioactive compounds are easier to be extracted with higher polar solvents.

4.2 Total Phenolic content (TPC)

The total phenolic compound was determined by Folin Ciocalteu (FC) reagent method with modification as described in Baba & Malik (2015), where gallic acid is used as standard phenolic compound. The FC reagent contains phosphotungstic or phosphomolybdic acid complexes that transfer of electrons in alkaline medium from phenolic compounds (Singleton & Rossi, 1965). In the presence of phenols, it formed a blue chromophore which absorb light at 650 nm. The reaction temperature at 37℃ for one hour has been used to reduce the time necessary to attain the maximum colour.

The total phenolic contents were expressed in terms of gallic acid equivalent (the standard curve equation y = 0.0078x – 0.1482, R^2 = 0.963 where y = absorbance at 650 nm and x = concentration of total phenolic compound in µg/mL of the extract).

Figure 4.2 illustrate the gallic acid calibration curve together with its equation and R^2 value. Meanwhile, table 4.2 list the total phenolic content expressed in µg GAE/g of extract.

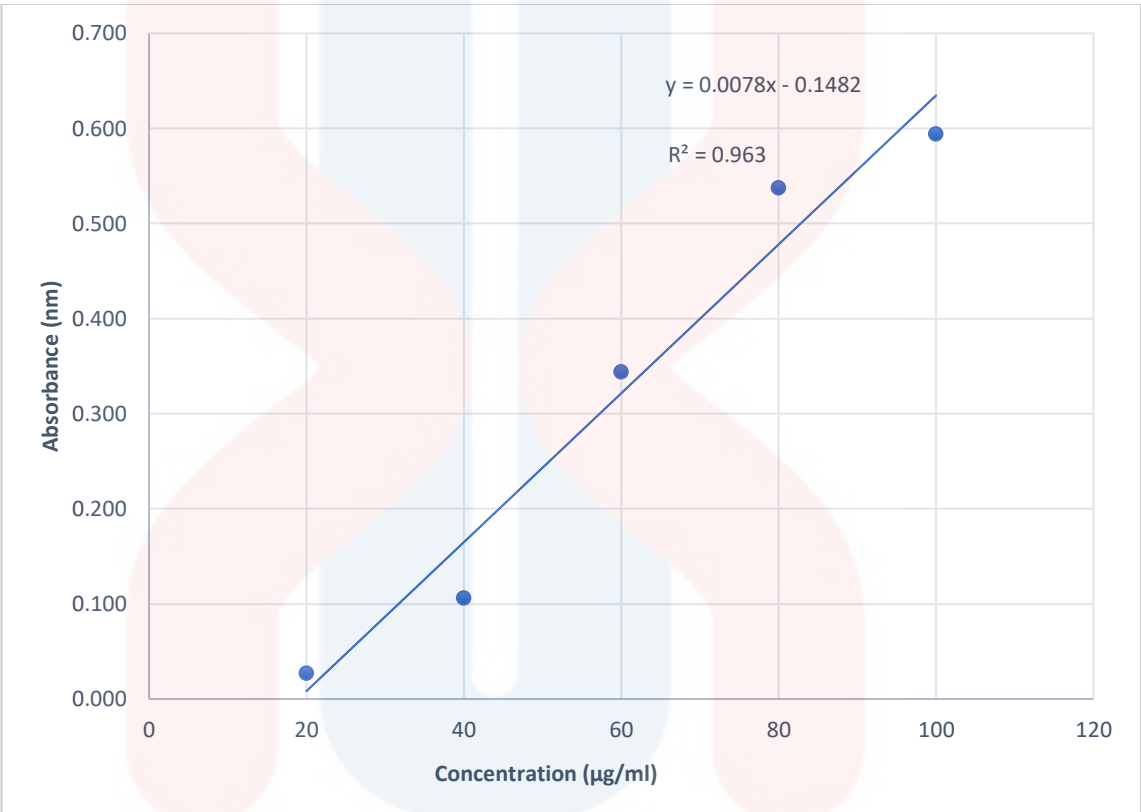

Figure 4.2: Standard calibration curve of Gallic Acid at concentration 20 to 100 µg/ml.

Table 4.2: Total phenolic content (µg GAE/g of extract)

Solvent	**Total Phenolic Content (µg GAE/g of extract)**
Methanol	48.615 ± 0.005
Ethanol	43.359 ± 0.007
Ethyl Acetate	34.769 ± 0.004

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

Table 4.2 shows that methanol has the highest total phenolic content followed by ethanol at 48.615 and 43.359 µg GAE/g respectively. Ethyl acetate, a low polar solvent recorded the lowest total phenolic content at 34.769 µg GAE/g. This highlights that methanol, a high polar solvent can easily extract the phenolic compound in *Alocasia farisii*. According to Babbar, Oberoi, Sandhu, & Bhargav (2014), higher polarity solvent has better solubility for phenolic components present in plant materials. Similar findings were found in Amensour et al. (2010) of *Myrtus communis* leaves and berries where the total phenolic content as following order: methanol > ethanol > ethyl acetate. The result was further analysed by comparing the samples in column bar graph as shown in figure 4.3.

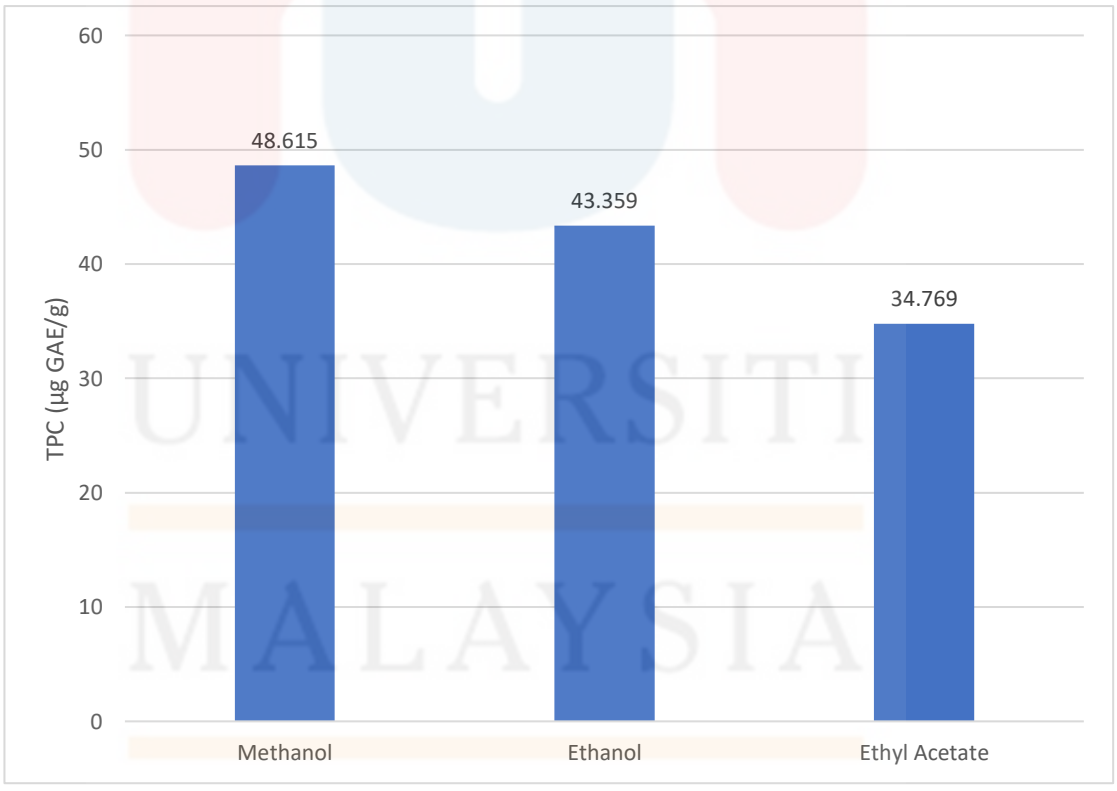

Figure 4.3 : Total phenolic content by various solvent extracts of *Alocasia farisii*

4.3 DPPH Radical Scavenging Assay

The antioxidant activity of *Alocasia farisii* was determined by using DPPH method as described in Do et al. (2014), with some modification where the concentration of DPPH is at 0.1 mM and varying concentration at 300,350,400,450 and 500 µg/mL. DPPH method makes use of the stable free radical DPPH, which has a strong purple colour that can be measured spectrophotometrically (Brand-Williams, Cuvelier, & Berset, 1995).

A freshly prepared DPPH solution exhibits a deep purple colour. When mixed with *Alocasia farisii*, it produced a fade violet colour as shown in Appendix B. According to Stankovic (2011), the presence of compounds that are capable of either donating hydrogen or transferring an electron, the DPPH will become discoloured as it probably converted into a bleached or colourless product like 2,2-diphenyl-1-hydrazine resulting decrease in absorbance at 517 nm. In the literature, the change in DPPH absorbance after the addition of sample is often used as an index of the antioxidant capacity of the sample (Holtz, 2009).

The antioxidant activity of three different extracts from the *Alocasia farisii* leaves and petioles is expressed in terms of percentage of inhibition (%) and IC50 values (µg/ml). Parallel the examination of the antioxidant activity by the plant extracts, the values of ascorbic acid as standard compound were obtained and compared to the values of the antioxidant activity. The calibration curve of ascorbic acid was constructed in Appendix C. The strength of different concentration by extract solution that donated hydrogen or transferring electron to the DPPH radical is shown in table 4.3.

Table 4.3: Antioxidant activity of plant extract as determined by DPPH assay

Solvent	Concentration of plant extract (µg/mL)	Antioxidant Activity (%)
Methanol	300	51.891 ± 0.003
	350	55.083 ± 0.002
	400	61.466 ± 0.001
	450	63.83 ± 0.004
	500	66.43 ± 0.004
Ethanol	300	51.182 ± 0.002
	350	54.374 ± 0.006
	400	58.156 ± 0.002
	450	60.165 ± 0.001
	500	64.894 ± 0.006
Ethyl Acetate	300	45.272 ± 0.001
	350	47.281 ± 0.001
	400	51.064 ± 0.004
	450	52.01 ± 0.002
	500	58.274 ± 0.007

*values were expressed as average ± standard deviation of triplicate measurements

As can be seen in the table 4.3, the antioxidant activity is directly proportional to the concentration of plant extracts. As the concentration of plant extract increases from 300 µg/mL to 500 µg/mL, the antioxidant activity also increases. Antioxidant activity varied from 45.272% to 66.43%. The highest antioxidant activity was obtained with the methanol (66.43%), followed by the ethanol (64.894%) and ethyl acetate (52.01%). Similar findings were found by (Stankovic, 2011), that methanol extract is

higher than ethyl acetate in *Marrubium peregrinum* L. (*Lamiaceae*). The result of antioxidant activity was further analysed in a column bar graph by comparing the different plant extract (300,350,400,450 and 500 µg/mL) as shown in figure 4.4.

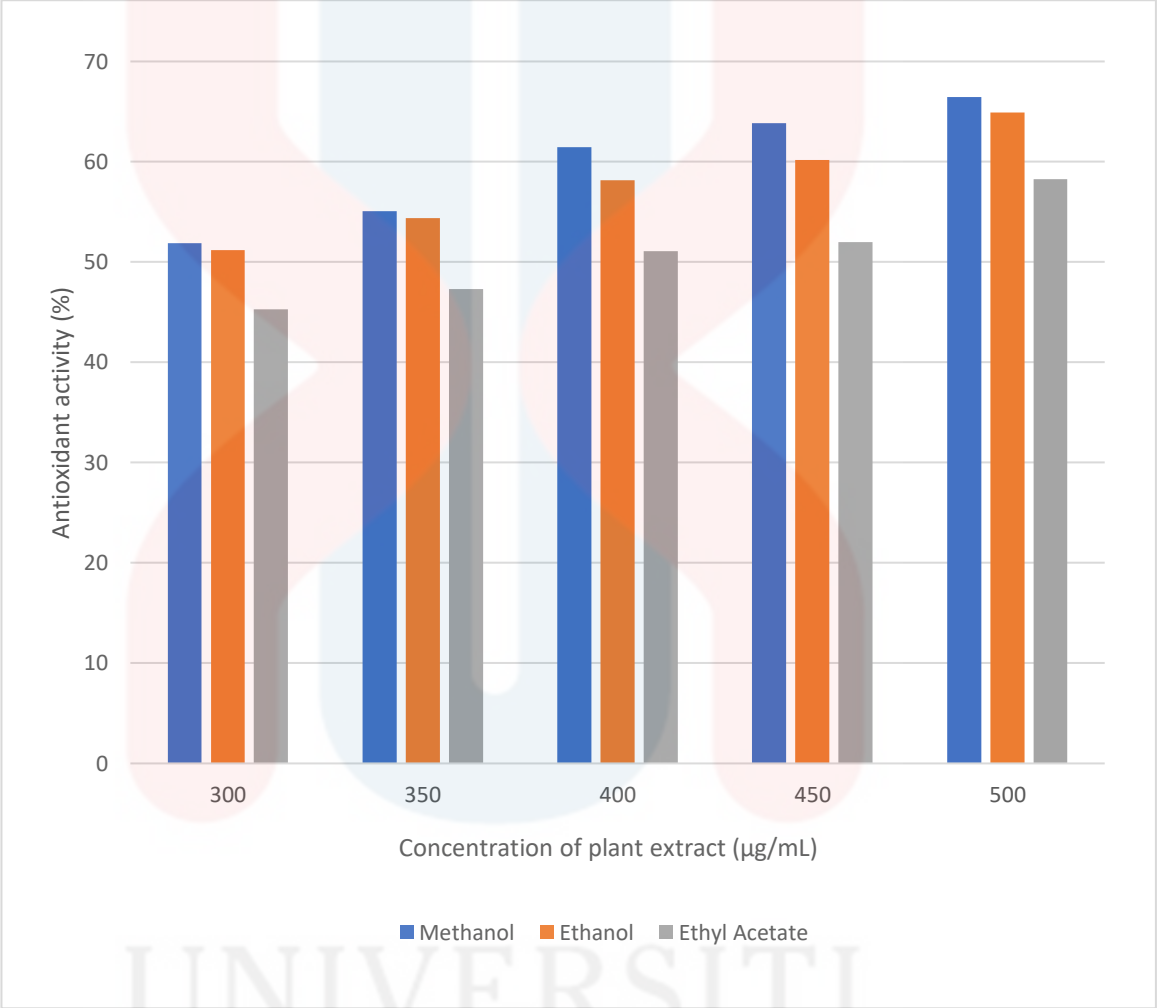

Figure 4.4: Comparison of antioxidant activity different plant extract as determined by DPPH assay.

Determination of IC_{50} value is important to know the amount of plant extract needed to decrease the absorbance of DPPH by half-maximal (50%) (Marxen et al., 2007). The IC_{50} value was determined through the equation received by graphically plotting the % inhibition versus concentration plot, using a non-linear regression algorithm in Microsoft Office Excel Software. The result of IC_{50} value obtained was further analysed in column bar graph as shown in figure 4.5.

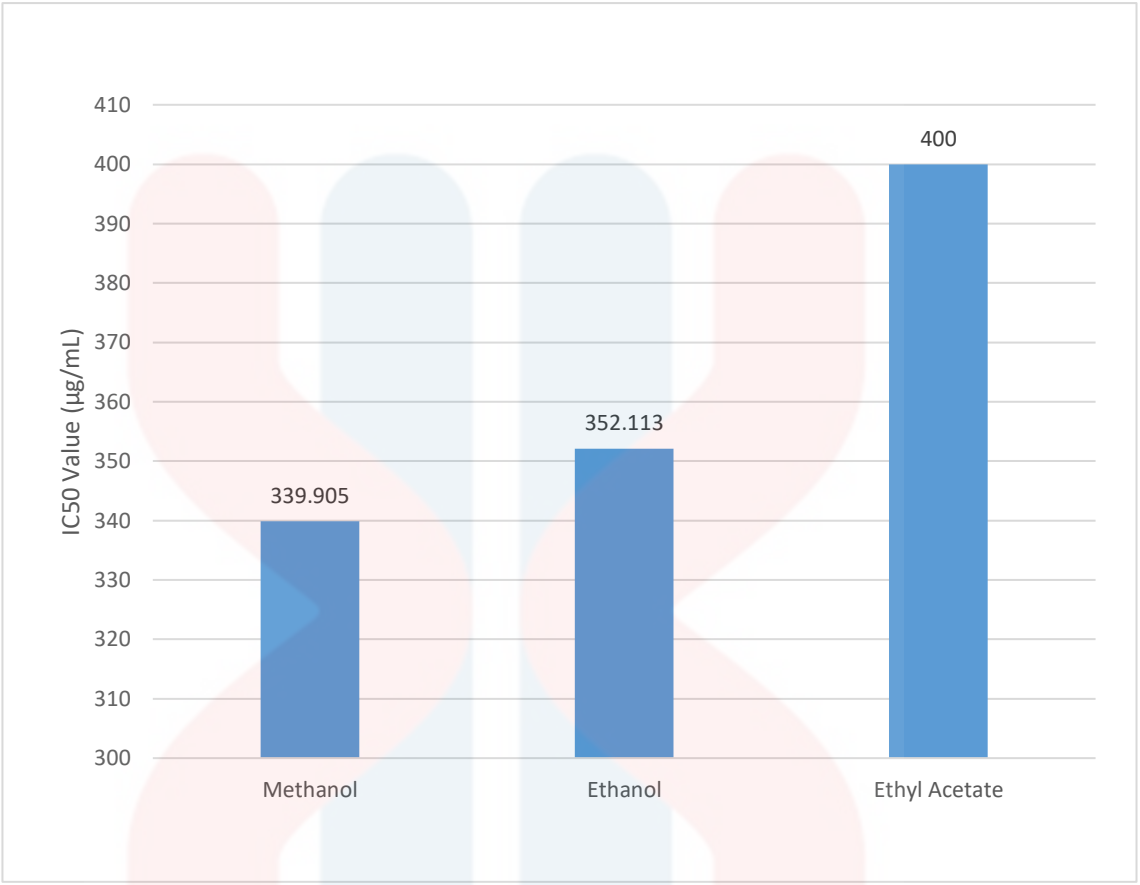

Figure 4.5*: IC_{50}* value in different plant extracts.

Based on figure 4.5, it can be seen that methanol has the lowest IC_{50} value, which means it have a high antioxidant activity as it can neutralized 50% of free radicals at the concentration of 339.905 µg/ml. A moderate activity was found in ethanol with IC_{50} value of 352.113 µg/mL and ethyl acetate recorded at highest with 400 µg/mL, indicating low antioxidant activity. This is parallel to the results obtained in table 4.3 and figure 4.4. Similar study by Zhang (2015) study in *Juglans regia L.* where the IC_{50} value as following order: methanol < ethanol < ethyl acetate. Besides that, Mandal et al. (2010) study on *Alocasia fornicata* (Robx.) Schott also shows IC_{50} value at 128.07 µg/ml in ethyl acetate extract which is lower than the values obtained in the present study.

4.4 Correlation between total phenolic content (TPC) and IC_{50} value of DPPH analysis.

The results showed that total phenolic content was significantly negative correlated with IC_{50} value of DPPH (r = -0.982). This shows that total phenolic content does not fully influence the antioxidant activity of *Alocasia farisii* plant extracts. Similar findings was found by Fidrianny, Suhendy, & Insanu (2018), where total phenolic content in tuber of sweet potato (*Ipomoea batatas)* were significantly negative correlated with IC_{50} value of DPPH. Besides that, a study using leaves extract of *Sechium edule* (Jacq.) Swartz also showed negative correlation between TPC and IC50 of DPPH scavenging capacity where r = -0.966 (Fidrianny, Ayu, & Hartati, 2015). Maisarah et al. (2013), note that not all antioxidant activity is due to phenolic content as it might be due to other various antioxidant compounds. Furthermore, phenolic does not incorporate necessarily to all the antioxidants that may present in the extracts.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

As a conclusion, the objectives of this study were achieved as it provides the total phenolic content and antioxidant activity of *Alocasia farisii* leaves and petioles. The high polar solvent which is methanol extracted the highest phytochemicals compared to other lower polar solvent. This highlights that most phytochemicals in *Alocasia farisii* are soluble in high polarity solvent. The results of this study provide useful information on the total phenolic content and antioxidant activity of *Alocasia farisii*, that can be a reference for further research on this species of *Araceae* family. Besides that, the leaves and petioles of *Alocasia farisii* may be exploited its beneficial as sources of natural antioxidant

5.2 Recommendation

In order to get a precise concentration, the micropipette must be calibrated and autoclave before used. Besides that, the micropipette tip must be in good condition. The fresh leaves and petioles must be extract immediately after sampling to get better results of total phenolic content and antioxidant activity of *Alocasia farisii*. Furthermore, the tuber can be used to get the whole total phenolic and antioxidant activity of the plant species. Further investigation for isolation and identification of the phytochemicals responsible for antioxidant activity can be also be conducted.

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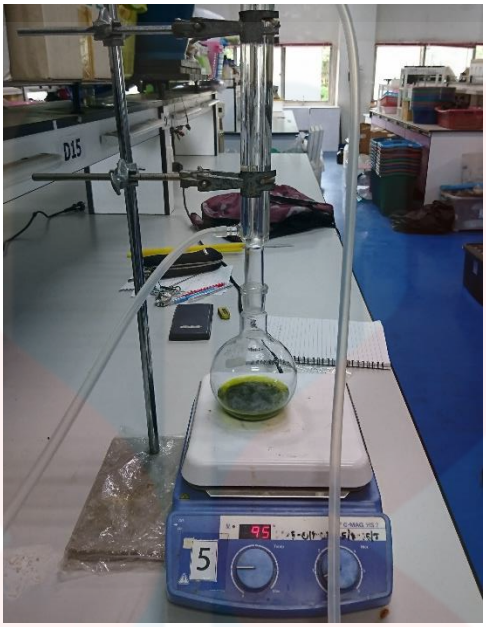
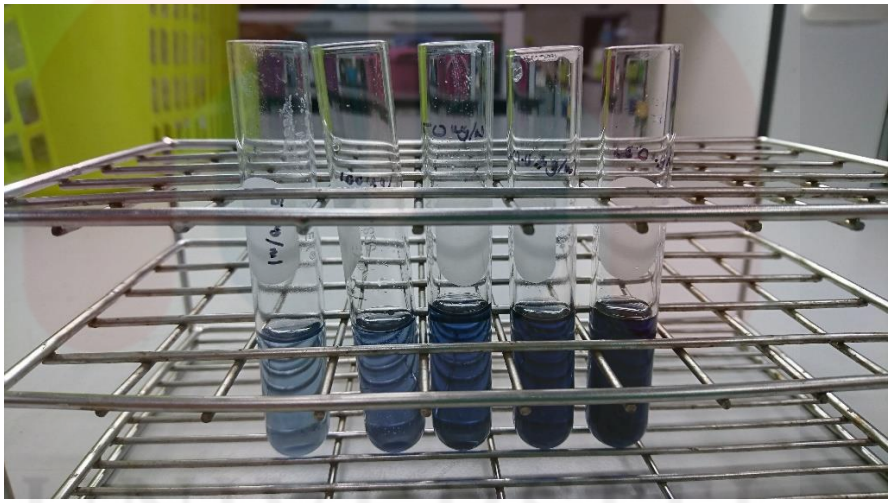
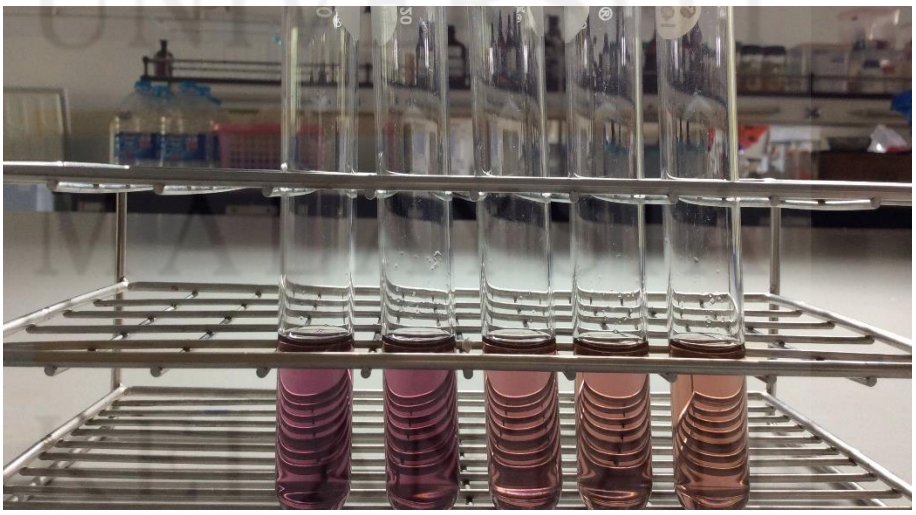
APPENDIX A

*Alocasia farisii***,** Gua Ikan, Kelantan.

APPENDIX B







Determination of total phenolic content and antioxidant activity of *Alocasia farisii* being carried out at the laboratory of Prince Songkla University, Suratthani.

APPENDIX C