DETERMINATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *ALOCASIA LONGILOBA* MIQ. (ARACEAE)

by

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DECLARATION

I declare that this thesis entitled "DETERMINATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *ALOCASIA LONGILOBA* MIQ. (ARACEAE)" 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 : Nur Izzati Binti Mohamad

Date : 17 January 2019

APPROVAL

"I/ We hereby declare that I/ we have read this thesis and in our 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"

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Date : ……..

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DETERMINATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *ALOCASIA LONGILOBA* MIQ. (ARACEAE)

ABSTRACT

Alocasia longiloba Miq belongs to Araceae family, which has the medicinal potential. The study on its antioxidant properties are important in order to know the active compounds. Currently, aroids medicinal properties are not widely known and there is lack of data regarding this species. In this study, the total phenolic content and antioxidant activity of *Alocasia longiloba* Miq. were determined by using UV-visible spectrophotometer. Methanol, ethyl acetate, and hexane were used in this study to extract the plant materials. From this study, it showed that the 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 antioxidant activity of *A. longiloba* Miq. can be expressed by using DPPH radical scavenging activity and the IC_{50} value for the three extracts were founded. Hexane extract had the highest percentage of DPPH radical scavenging activity while methanol extract was the lowest. *A. longiloba* Miq. has the potential antioxidant that can be used for the medicinal purposes.

KADAR TOTAL FENOL DAN AKTIVITI ANTIOKSIDAN DALAM *ALOCASIA LONGILOBA* MIQ. (ARACEAE)

ABSTRAK

Alocasia longiloba Miq. adalah spesies di bawah keluarga Araceae yang mempunyai potensi dalam bidang perubatan. Disebabkan banyak tumbuh-tumbuhan yang boleh digunakan untuk tujuan perubatan, kajian terhadap sifat-sifat antioksidan dalam tumbuhan ini amat penting bagi mengetahui kandungan sebatian semula jadi di dalam tumbuhan tersebut. Pada masa ini, kajian terhadap spesies ini kurang dilakukan menyebabkan kekurangan informasi terhadap spesies ini. Tujuan kajian ini dijalankan adalah untuk menentukan kandungan fenol total dan aktiviti antioksidan di dalam *A. longiloba* Miq. dengan menggunakan tiga pelarut yang berlainan bagi proses mengekstrak. Spektrofotometer digunakan dalam kaedah ini. Metanol, etil asetat dan heksana digunakan dalam kajian ini bagi mengekstrak sebatian di dalam tumbuhan. Kajian ini menunjukkan bahawa ekstrak etil asetat mempunyai kandungan fenol total yang tertinggi iaitu 46.013 mg GAE/g diikuti dengan ekstrak metanol (32.936 mg GAE/g) dan ekstrak heksana (31.782 mg GAE/g). Aktiviti antioksidan di dalam A. longiloba Miq ini dapat diekspresi kan melalui kadar aktiviti pemotongan radikal dan nilai IC_{50}. Ekstrak heksana mempunyai kadar aktiviti pemotongan radikal yang paling tinggi manakala ekstrak methanol adalah yang paling rendah sekali. Kesimpulannya, *A. longiloba* Miq. mempunyai kandungan antioksidan yang berpotensi untuk digunakan dalam bidang perubatan.

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A. longiloba Miq.	*Alocasia longiloba* Miq**.**
GC-MS	Gas Chromatography-Mass Spectrometry

CHAPTER 1

INTRODUCTION

1.1 Background of Study

"Keladi Candik, Keladi Candik Merbah and Keladi Ular" are the local names of *Alocasia longiloba* Miq which belongs to Araceae family. *A. longiloba* Miq is native species in Peninsular Malaysia, China, Laos, Thailand, Borneo, Sumatera, and Sulawesi (Mashhor et al., 2012). It is traditionally used for remedies, the bioactive compounds and antioxidants in the plants are good in treating the wounds. However, there is restricted data on *A. longiloba* Miq. since there is lack of study on this species.

Other than that, the selection solvent for the extraction is very crucial as not all of the natural compounds can be extracted by using the same solvents. The polarity of solvent is important in order to produce the maximum yield of extraction and also to extract the bioactive compounds. Thus, this study will give the information regarding to the most suitable solvent to be used in the extraction method in order to extract the natural compounds in the extract of *A. longiloba* Miq..

1.2 Problem Statement

The medicinal plants play an important role in human health care as traditional treatment since ancient days. Currently, aroids medicinal properties are not widely known. Because of that, they prefer to use modern medicine instead of traditional herbal plant to cure some illness. *Alocasia longiloba* Miq. also known as "Keladi Candik" by its local name, is easily found through Malaysia (Appendix A). However, many still do not know about the benefits and its medicinal properties. Further research on this species should be carried out to conserve the knowledge and to evaluate the beneficial antioxidant properties in *A. longiloba* Miq. Hence, the uses of modern medicine can be reduced by replacing it with natural herb medicine.

1.3 Objectives

The objectives of this study are:

1. To determine the total phenolic content in *Alocasia longiloba* Miq. with different extract solvents .

2. To determine the antioxidant activity in *Alocasia longiloba* Miq. by using DPPH method with different extract solvents.

1.4 Scope of Study

Plants have been a source of nutrition, medicines and crop protection agents for centuries. It is believed that over 80% of all medication taken in the developed world is derived from plant origins. This study focused on the determination of total phenolic content and antioxidant activity in *Alocasia longiloba* Miq., which related in medicinal area. The plant petioles and leaf blades are the parts of plants that were used for the extraction method. The plant materials were collected and dried before the extraction process begin. Only 3 solvents with different polarities were used in the extraction process, which are methanol, ethyl acetate and hexane. The purpose of using the different solvents is to know the most solvent which is more efficient in giving the result based on the different polarities. The samples were analyzed by using UV-Visible Spectrophotometer.

1.5 Significance of Study

Study of plant *Alocasia longiloba* Miq. could give many benefit to the societies, researchers, entrepreneurs, product developers, and others. Firstly, this research would give chance to the societies to become more knowledgeable about *A. longiloba* Miq which then can be used as therapeutic traditional treatment as an alternative to the modern medicine.

The study also give benefit to the researcher. This is because lack of study on this plant causing minimal or no data on the issues related to *A. longiloba* Miq.

Lastly, the product developer also will benefit from this study, especially those who are involve in medicinal sectors could launch the new products based on the *A. longiloba* Miq.

CHAPTER 2

LITERATURE REVIEW

2.1 General of Araceae

The family of Araceae, also known as aroid, consists of 105 genera and more than 3300 species have been identified. Mostly, they are herbaceous and diverse in its morphology, their attractive leaves make them a well-known recognized feature by the researchers (Chen J. et al., 2007). Furthermore, Araceae is the fourth largest family of monocotyledons after orchids, grasses, and sedges. This is one of the most crucial mesophytic plant families (Mashhor et al., 2012). Naturally, the family exists in every region in the world except the Antarctic. The humidity of the area for the growth of aroids are very important as they depend on water to grow. According to (Mashhor et al, 2012), Araceae family are cannot adapted in cold and arid conditions otherwise those are geophytes.

However, it is predominantly found in tropical rainforest in Malaysian Peninsular and also throughout tropical forest in Asia. Furthermore, about 10 genera only extend into

northern temperate zones while no species are observed in the Southern hemisphere that undergoes temperate climate (Grayum, 1990).

2.2 Uses of Araceae

According to previous studies on aroids, they are consist of beneficial medicinal value that can heal some diseases in traditional medicines. The bioactive compounds and antioxidants such as phenolic, tannin, and flavonoid are good in the medicinal area. Based on the research in (Frausin *et al*., 2015), *Anthurium schott* and *Philodendron schott* are effective in the treatment of headaches, malaria, fevers and liver problem, while *Aglaonema treubii* is potentially in remedial agents for diabetes type 2 and HIV-1 infection (Chen *et al.*, 2007). Many more aroids need to be studied since some of them show high medicinal potential which can reduce the dependence on modern medicine.

Aroids also can be food resources for human and animals. Xanthosoma and Colocasia are under genera of Araceae which are the most popular of food aroids. Most of them are poisonous when fresh, however it can be eaten after being cooked (Mayo *et al*., 1997). Based on the previous study, inflammation on mouth, lips and throat will occur if the people do not cooked the edible aroids well. This is because of the present of needle-like raphids of calcium oxalate causing the biological reactions (Bradbury & Nixon, 1998). For the example, *Colocasia esculenta* and *Xanthosoma Sagittifolium* are the popular edible aroids among Malaysians.

The diversity and fascinating Araceae leaves making aroids one of many popular ornamental plants (Mayo *et al.*, 1997). Aroids can be sold and display for their aesthetical uniqueness. According to (Henny *et al.*, 2004), *Aglaonema, Dieffenbachia, Philodendron and Syngonium* are the example of ornamental foliage plants, which are the major components of United States foliage plant industry and can generate income for the locals.

2.3 *Alocasia longiloba* Miq.

There are over 113 of plants under genus Alocasia that grows in Malaysian region, Southeast Asia, and Australia (Nauheimer *et al*., 2012). *Alocasia longiloba* Miq is one of the species of *Alocasia*. According to (Park, 2013), it is similar to *Alocasia lowii, Alocasia denudate, Alocasia veitchii, Alocasia singaporensis* and *Caladium veitchii*. This species can be found in Central Vietnam, Malaysian Peninsular, Thailand, Sumatra, Borneo, Java, and Sulawesi (Hay, 1998). It is integrated with *A. lowii* which typically has broader leaf blades (Boyce, 2008; Hay, 1998).

Furthermore, *A. longiloba* Miq. has unique morphological characteristics that should be investigated. Commonly, this species are less than 1.5 m tall and the leaves are separated to 3 together for a plant. The petioles are dark green to chocolate colour and it has rough structure. While the colour of blades are dark green and has narrowly triangular shape. *A. longiloba* Miq also are highly uniform inflorescence morphology, fibrous cataphylls, and have purple backed and white veined blades (Wulandari *et al*., 2008). They

usually grow in rain forest with shady area, having good humidity, and high humus content.

2.4 Medicinal Plants

Plants are playing important roles to the environment and also to human health. The active ingredients can be extracted from the plants and become a valuable source in maintaining human health. In fact, about 80% of individuals from developed countries preferred to use the traditional medicine, which is from medicinal plant that contains of beneficial compounds (Nascimento *et al.*, 2000). Hence, the plants should be further studied to better understand their properties, safety, and effectiveness.

According to (Jain *et al.*, 2011), there are many types of plant species that can be used as source of medicines. About 38 species out of 51 wetland edible plant species in Bhutan are used in medicinal purposes by the local. The contents of bioactive compounds in each parts are not identical for all plant species. Based on Jain's study, there are 15 species which the whole plant is used for medicinal purpose, while 14 other species only requires parts for the therapeutic medicine. The plants are able to cure the diseases of cuts and injuries, cough and fever, blood pressure problem, wounds, and also diabetes. In addition, *Colocasia esculenta* is believed to be able heal cuts and injuries by using its leaves and tubers (Goncalves *et al*., 2013). Hence, it approved that many of plants species have medicinal value that could be used by human for medicinal purpose.

The compounds that contained in medicinal plants are useful in therapeutic agents. Most of medicinal compounds or secondary metabolites can be obtained from the plants. Alkaloids, coumariness, terpenoids, xanthones, quinones, flavonoids, and phenolics are the natural products that are very important in medicine (Amoa Onguéné *et al.*, 2013). In addition, artemisinin which is sesquiterpenoid, is very powerful in combatting the malaria disease (Yang *et al.*, 2016). Hence, study on medicinal plants are very crucial as the research can gain the knowledge on the usefulness of plants.

2.5 Antioxidant Properties in Plant

An antioxidant can be described as any substances that can postpone or stop the oxidative damage to a target molecule. The ability of antioxidant to trap free radicals become the main characteristic in order to inhibit the oxidative system that lead to deteriorating disease (Mahdi-Pour *et al.*, 2012). As mentioned by (Saeed, Khan, & Shabbir, 2012), free radical can be deactivated by antioxidants before it attacks the target biological cells. There are a lot of studies have been done to investigate the antioxidant properties in the medicinal plants.

Natural antioxidants are more preferable as it is very effective to inhibit the destructive processes caused by oxidative stress. Other than that, it is safer as it produced by plant rather than synthetic counterparts. (Loganayaki, Siddhuraju, & Manian, 2013)

stated that the use of artificial antioxidants is an old practice and the status on its safety become doubted by the consumers.

Nowadays, most of the consumers and researchers are interested in medicinal plants especially on its antioxidant activity, which is beneficial to human health. The natural antioxidants from plants such as phenolic and flavonoids had been utilized commercially as antioxidant additives or nutritional supplements. However, this process can be accomplished via making a lot of research on medicinal plant to get more information on antioxidant potential of the plant species. As they are safer and contained bioactive compounds, studies on determining the possible plants with antioxidant potential were carried out more by researcher (Patel, Patel, & Kajal, 2010).

According to (Balasundram, Sundram, & Samman, 2006), phenolic compounds are derived from pentose phosphate, shikimate, and phenylpropanoid pathways in plants, which are secondary metabolites. As noted by (Dhami, Reddy, & Mukherjee, 2012), phenolic compounds are divided into two components which are phenolic acids and polyphenols. These compounds are linked with mono and polysaccharides, which can appeared with one or more phenolic group or can develop as components like ester or methyl ester. It showed wide range of physical properties such as anti-allergenic, anti-inflammatory, anti-artherogenic, anti-microbial, anti-thrombotic, cardioprotective, vasodilatory effects and antioxidant.

Moreover, redox properties are the most important in the antioxidant activity of phenolic compounds which let them to be a reducing agent, hydrogen donator, singlet oxygen quenchers and metal chelation potential (Balasundram *et al*., 2006; Dhami *et al*., 2012). Structurally, phenolic compounds consist of an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds.

Table 2.2 Classes of phenolic compounds

Classes	Structure
Simple phenolic, benzoquinones	C_6
Hydroxybenzoic acids	$C_6 - C_1$
Acethopenones, phenylacetic acids	$C_6 - C_2$
Hydroxycinnamic acids, phenylpropanoids (coumarins, isocoumarins, chromones, chromenes)	$C_6 - C_3$
Napthoquinones	$C_6 - C_4$
Xanthones	$C_6 - C_1 - C_6$
Stilbenes, anthraquinones	$C_6 - C_2 - C_6$

Flavonoids, isoflavonoids	$C_6 - C_3 - C_6$
Lignans, neolignans	$(C_6 - C_3)_2$
Biflavonoids	$(C_6 - C_3 - C_6)_2$
Lignins	$(C_6 - C_3)_n$
Condensed tannins (proanthocyanidins or flavolans)	$(C_6 - C_3 - C_6)_n$

Source :Balasundram et al. (2006)

The selection of solvent for extraction process is important in order to isolate the antioxidants, determining the extraction yield, total phenolic content and antioxidant activity. These mechanism depends on the solvent which have different antioxidant potentials with different polarity (Arakrak, 2014). Do *et al.* (2014) indicated that the extraction method and the solvent used for extraction effect the extraction yield and antioxidant properties. The chemical characteristics and polarities of antioxidant compounds affecting the solubility of compounds in the particular solvents. Usually, polar solvents are used for reclaiming the polyphenols in plant.

Methanol, ethanol, acetone and ethyl acetate are amongst the most effective solvents for extraction of antioxidants and phenolic compounds. According to (Razali et al, 2012), methanol is the best solvent to extract the compounds in numerous parts of *T.*

indica plant. This is also supported by (Sultana, Anwar, & Ashraf, 2009), and noted that methanol and ethanol are the best solvent to extract the antioxidant compounds.

There are many methods or techniques in order to extract the medicinal compounds in plants. Some compounds are very reactive to heat and oxygen so that more care should be taken to avoid the compound's loss from the oxidation process. According to (Aspé & Fernández, 2011), maceration, soxhlet extraction, and percolation are the traditional method to extract the plant materials. These methods are quite slow, require a large quantity of solvent, and sometimes the active compounds are easy to deteriorate. Alternatively, microwave-assisted extraction (MAE) and ultrasound assisted extraction are also used to extract compounds as they are more rapid and efficient. Nevertheless, according to (Sultana *et al.*, 2009), the maximum yield of extraction can be obtained under reflux extraction.

In addition, the selection on the technique used in detection of antioxidant activity is very important as it gives different results based on different techniques. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate), FRAP (ferric reducing antioxidant potential), and ORAC (oxygen radical absorption capacity) are the various techniques to determine the antioxidant activity in plant extracts (DUDONNE, 2009). Furthermore, Cai *et al*. (2004) claimed that DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate) are the most frequent and reliable techniques in order to determine the antioxidant activity. This is because of these methods are more stable and gives the rapid result. (Hamid *et al.*, 2013), also noted that DPPH assay is manageable as

well as sensitive yet easy to handle which is one of the advantages rather than using the other methods.

Even though there are many methods to determine the total phenolic content in plant extract, the Folin-Ciocalteu is one of the methods that usually used by researcher. The redox reagent (Folin-Ciocalteu reagent) can be reacted with polyphenols in plant extract by forming blue color which can be measured by UV-visible light spectrophotometry. Besides, it provides the exact and precise data for some groups of phenolic compounds. This is due to the reaction between the compounds that may change its color because of the differences in unit mass and reaction kinetics (Blainski, Lopes, & De Mello, 2013).

Other than that, the standard and blank are important to measure the antioxidant activity and total phenolic content. The color of blank solution for phenolic content changed from yellow to colorless as the Folin-Ciocalteu reagent is not stable under alkaline conditions. This is because of the reaction between the solutions with all the phenols before it is demolished. The standard solution is very crucial in order to determine the antioxidant activity and phenolic content as it will be the reference for the experiment's result. Ascorbic acid is used as the standard solution for the free radical scavenging activity (Molyneux, 2003). The common compounds used as the standard for the phenolic content are Gallic acid, tannic acid, catechin, tyrosine and so forth. Additionally, Gallic acid is the more frequently used as the standard and the results are described in milligram Gallic acid equivalents (GAE) per liter (Lamuela-ravents, 1999). A study have been done in determining the anti-diabetic potency of medicinal plants in Indonesia. The anti-

diabetic potency, antioxidant activities, phenolic and flavonoids were provided in the results. The content of phenols and flavonoids were discovered by using Gallic acid and quercetin as a standard, respectively (Ratnadewi *et al.*, 2018).

Furthermore, based on the (Kanthal *et al.*, 2014), gas chromatography-mass spectrometry (GC-MS) analysis of the whole plant methanol extract of *Lactuca runcinata* showed the presence of some bioactive components in the plants which have potential as a source of natural herb medicine. Nevertheless, UV-visible spectrophotometry method is generally used to determine the colorimetric reaction. According to (Blainski *et al.*, 2013) this method is more practicable, less time consuming and cheap. Hence, the pharmacology potency in the potential medicinal plants can be known by using these methods.

CHAPTER 3

METHODOLOGY

3.1 Materials

Apparatus

Weighing scale, mechanical blender, beaker, test tube, glass rod, conical flask, round bottomed flask, measuring cylinder, hot plate, condenser, refrigerator, tissue paper, cuvette, micropipette, micropipette tip, centrifuge tube, test tube rack, aluminium foil, Whatman-filter paper, UV-visible spectrophotometer

Chemicals

Methanol, ethyl acetate, hexane, ethanol, Folin-Ciocalteu reagents, ascorbic acid, Gallic acid, sodium carbonate, deionized water

3.2 Methods

3.2.1 Collection and processing of *Alocasia longiloba* Miq.

The plants of *Alocasia longiloba* Miq. were collected at Kampung Tiong Dalam, Kota Bharu, Kelantan, Malaysia. The petioles and blades of the plants were washed with tap water and dried it in open air. Then, the plant materials were chopped to small pieces and baked in the oven for 5 days at 50ºC. After drying process, the plant materials were grounded well by using mechanical blender until the plant material become fine powder. The fine powder was saved in air-tight containers for the next analysis. Refer Appendix B.

3.2.2 Preparation of plant extracts

According to (Ghasemzadeh & Jaafar, 2014), the method was slightly modified. 3.5 grams of fine powder were weighed by using electronic scale. Then, the weighed fine powder was extracted in 45mL of methanol, ethyl acetate, and hexane respectively under reflux extraction. Each mixture was added in the round bottomed flask and the flask was attached with reflux condenser on top of the round bottomed flask. Next, the tube was attached to the condenser for the water flows. The liquid forming in the condenser and flowing back into the reaction was observed (Appendix B). Then, the mixture was heated for 4 hours with the various temperature (50-70ºC). After 4 hours, the solutions were allowed to cool at room temperature. The extracts were separated from the residues by filtering through Whatman No. 1 filter paper (Sultana *et al.*, 2009). The extract was concentrated in vacuum using rotary evaporator to 10% of the original volumes. The

temperatures of rotary evaporator were set to 40ºC. Then, the extract was kept in refrigerator for next analysis.

3.2.3 Percentage yield of extract

The percentage yield of extract was obtained by using this formula:

$$\text{Percentage yield (\%)} = (a/b) \times 100 \quad (3.1)$$

Where, a = the dry weight of extract

b = initial weight sample

The yield of extraction was calculated for each solvent (Patakas et al., 1988) .

3.2.4 Total Phenolic Content

Total phenolic contents (TPC) was determined by using Folin-Ciocalteau (FC) assays. The crude extract was dissolved with ethanol to a concentration 1mg/mL. 200µl of diluted extract was taken and the volume was adjusted until 3ml with deionized water. Then, the mixture was mixed thoroughly after 0.3ml of Folin Ciocalteu reagent was added into it. The mixture was shook for 3 minutes (Baba & Malik, 2015). After that, 2 mL of 20% of sodium carbonate (Na_2CO_3) was added to the mixture and were incubated for 1 hour (Dhawan & Gupta, 2016). Lastly, the mixture was poured into cuvette and the

absorbance was measured by using UV-visible spectrophotometer at 765 nm (Siddiqui *et al,*. 2017). Blank was prepared by switching the crude extract solution into ethanol.

Preparation of standard solution of Gallic acid

The calibration curve was made up by using standard solution Gallic acid. Stock solution of Gallic acid was made up into 1mg/mL. The stock solution was diluted into concentration of 20, 40, 60, 80, 100 µg/mL of Gallic acid solutions. 200 µL of each concentration was taken and the procedure in determining the total phenolic content was repeated to construct the calibration curve. Based on the measured absorbance, the concentration of total phenolic were determined from the calibration curve (Stankovi, 2011).

3.2.5 DPPH Radical Scavenging Activity

The antioxidant activity of the extract will be determined by DPPH method. DPPH was dissolved in ethanol to get concentration of 0.1mM (Maizura, Aminah, & Aida, 2011). 2.5ml of extract with different concentration of 300, 350, 400, 450 and 500µg/ml will be mixed respectively with 2.5ml of DPPH solution in a test tube. Then, the test tube was incubated in the dark for 20 minutes at room temperature before the absorbance will be measured by using UV-visible spectrophotometer at 517nm (Do *et al.*, 2014). According to (Razali *et al.*, 2012), the percentage of radical scavenging activity was calculated by using the formula:

$[(A_{control}-A_{extract})/(A_{control})]\times100$ (3.2)

Where: $A_{control}$ is the absorbance of DPPH solution without extract

A_{sample} is the absorbance of sample with DPPH solution

Ascorbic acid was used as reference standard by dissolving it in ethanol to make the stock solution with the same concentration (1mg/mL). The control was prepared by mixing 2.5mL of ethanol with 2.5mL of DPPH solution. Blank was prepared by using ethanol. Then, the half-maximal inhibitory concentration (IC_{50}) was calculated (Nićiforović *et al.*, 2010).

3.3 Data Analysis

In this study, the result were expressed as mean ± standard deviation. The experiments were carried out in triplicate and the data in all the experiments were analyzed by using spreadsheet software (Aliyu et al., 2013).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Total extraction yield

Extraction is the first step in order to analyze the active compounds in plant materials. The selection of the solvent and the method for extraction were very crucial as it affects the ability and the effectiveness of the pharmacological activities. In the present study, the yield of extraction was studied by using 3 solvents with different of polarities which were methanol, ethyl acetate and hexane (Table 4.1).

Table 4.1 The extraction yield of plant extracts

Solvent	Total Extraction Yield (%)
Methanol	24.00
Ethyl acetate	3.14
Hexane	2.00

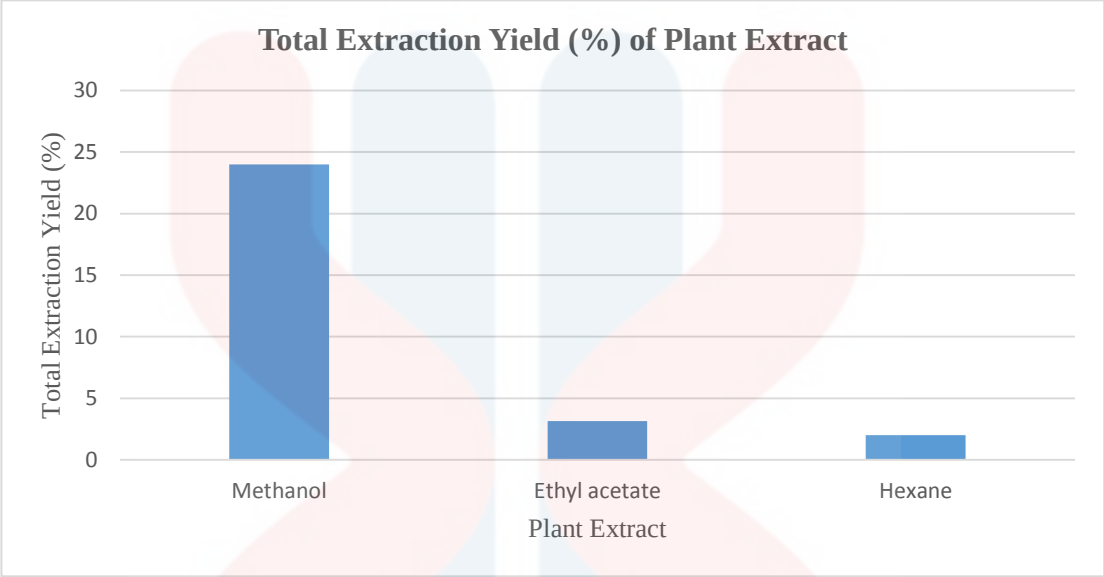

Figure 4.1 The total extraction yield of different plant extract.

Based on the Figure 4.1, it shows the percentage of extraction yield of plant extracts in different extract solvent. The highest percentage of extraction yield was observed by using methanol (24%). Hexane had the lowest percentage of extraction yield with 2% and followed by ethyl acetate with 3.14%. From this study, it shows that the extraction yield increased when the polarity of the solvent increased.

According to (Muhamad *et al.*, 2014), the polarity of solvents have important roles in extraction process in order to increase the solubility of the antioxidant compounds. From this study, it revealed that hexane was found as the least powerful to extract the bioactive compounds as it had low polarity index and give the lowest extract yield. Nevertheless, Abarca-Vargas, Peña Malacara, & Petricevich (2016) analyzed that, methanol and distilled water give the maximum extraction yield as they were more polar

than hexane. Hence, methanol is the most suitable solvent to be used as it give the maximum extraction yield in this study.

4.2 Total Phenolic Content

Phenolic which are secondary metabolites of plant have got attention by researcher as they are known as sources of potential natural antioxidants. This is because of their capable to be radical scavengers and metal chelators (Olugbami, Gbadegesin, & Odunola, 2015). The total of phenolic content was determined by using Folin-Ciocalteu method and used standard solution of Gallic acid as a reference. This method was commonly used in order to determine the phenolic content in the plant extract as it is simple, fast and convenient. The absorbance values of the extract solution were calculated in order to know the content of phenolic in the plant extract (Hossain & Shah, 2015). The value of total phenolic content in the plant extract indicates that the extract is contained a lot of phenolic compounds.

The content of phenolic was determined by using linear equation from the standard calibration curve of Gallic acid. Y = 0.0078x-0.1489 was the equation from the standard curve while the value of R^2 was 0.9632 (Figure 4.2). The phenolic content in plant extract was expressed as milligram GAE equivalent per gram extract and it was listed in Table 4.2 and showed in graph in Figure 4.3.

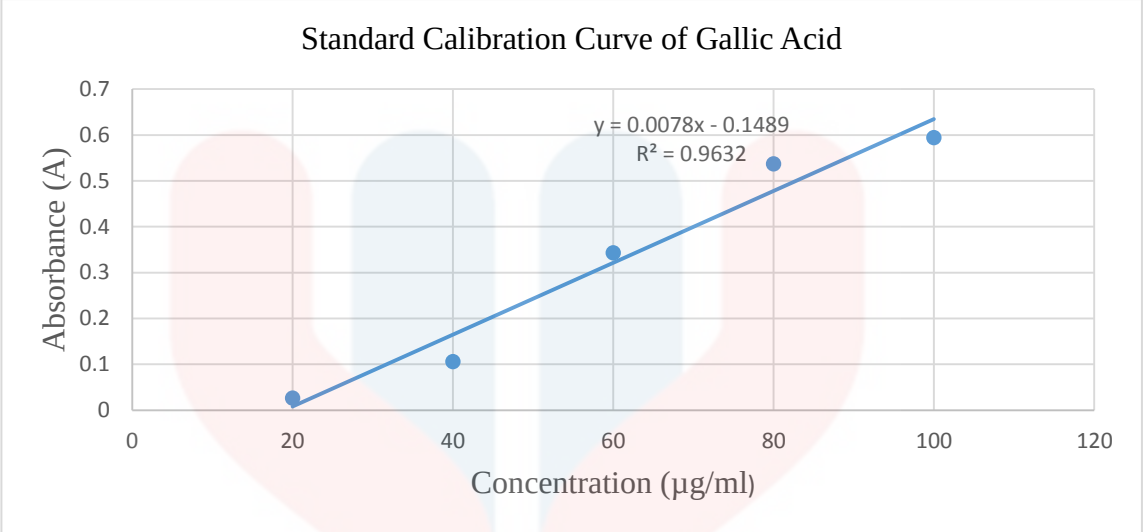

Figure 4.2 The standard calibration curve of Gallic acid

Based on the Table 4.2, there were various value of phenolic content in *A.longiloba* Miq.in different plant extract. The content of phenolic in plant extract with methanol, ethyl acetate and hexane was 32.936±0.0006, 46.013±0.0010 and 31.782±0.0006mg GAE/g respectively. Ethyl acetate extract was the highest content of phenolic followed by methanol and hexane.

Table 4.2 The total phenolic content of plant extract

Solvent	**Total Phenolic Content (mg GAE/g extract)**
Methanol	32.9362±0.0006
Ethyl acetate	46.0125±0.0010
Hexane	31.7823±0.0006

Each value in the table is represented as mean±standard deviation (n=3).

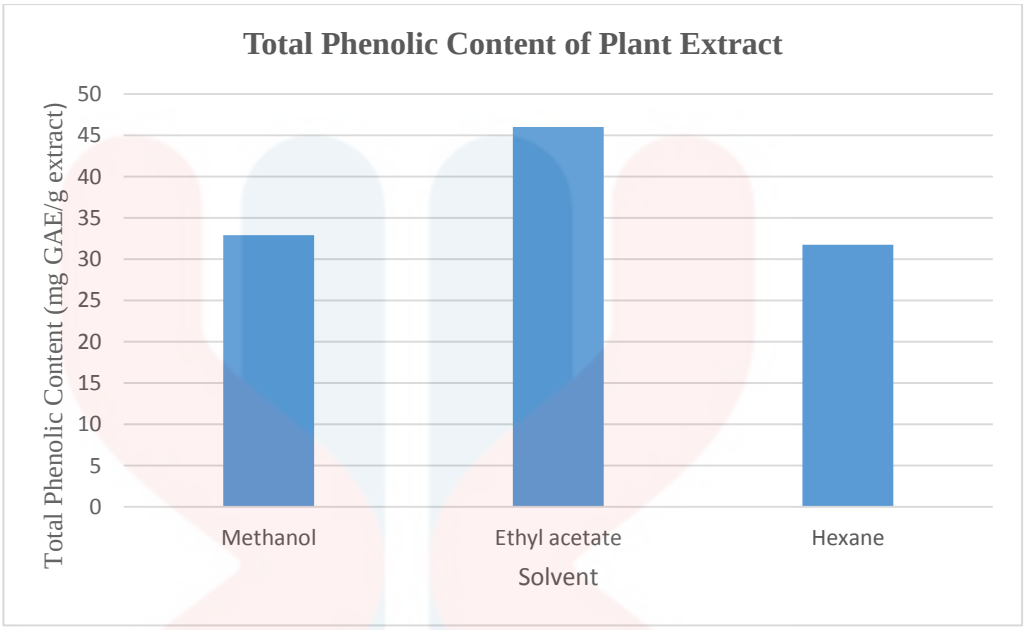

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Figure 4.3 The total phenolic content of plant extract

In the present study, the total phenolic content in ethyl acetate extract was the highest compared to methanol and hexane. This shows that ethyl acetate which is polar compound can easily extract the phenolic compounds in *A. longiloba* Miq.. Oliveira et. al (2012) stated that the phenolic contents in *Sidastrum micranthum* and *Wissadula perriplocifolia* showed the highest in ethyl acetate extract rather than aqueos, chloroform, hexane, n- butanol and dichloromethane. The phenolic content in methanolic extract more lower than ethyl acetate even though the polarity of methanol is more polar than ethyl acetate. It showed that the determination of the value of phenolic content is depend on their chemical reducing capacity relative to Gallic acid, not just through the measurement of phenolic compounds (Hossain & Shah, 2015).

According to (Tawaha et al., 2007), they said that, the present of total phenolic compounds in an extract is not specific to polyphenols. The other compounds could be

reacted with the Folin- Ciocalteu 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.

4.3 Antioxidant Activity

Antioxidant activity of *A. longiloba* Miq. extract can be determined by using DPPH radical scavenging activity. The DPPH is a stable free radical with a maximum absorbance at 517nm and it can easily undergo scavenging by an antioxidant (Loganayaki *et al.*, 2013). The degree of color changes of the solution was point out the scavenging potential of the extract. In addition, the loose of the absorption from the acceptance of electron or free radical species, causing discoloration of solution from purple to yellow.

As DPPH can be used in many samples with less time consumption and able to identify the bioactive compound at low concentrations, make it as an advantages in using this method (Esmaeili *et al.,* 2015). This method was utilized according to (Do *et al.*, 2014) with slight modification of the solvent and its concentrations. In this study, the free radical scavenging activity in the plant extract with different concentrations were evaluated and the result was shown in Table 4.3.

Based on Table 4.3 it showed the relation between types of solvent, concentration of solution, absorbance of the extract at 517nm by using UV-Vis spectrophotometry and the DPPH radical scavenging activity. From this study, the range of radical scavenging activity from 3 different extracts were between -2.4823±0.0006 % to 77.4232±0.0012 %. 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.

Table 4.3 The DPPH radical scavenging activity of plant extract and the absorbance at 517nm

Solvent	**Concentration (µg/ml)**	**Absorbance (A)**	**DPPH Radical Scavenging Activity (%)**
Methanol	300	0.867	-2.4823±0.0006
	350	0.758	10.4019±0.0012
	400	0.677	19.9764±0.0010
	450	0.662	21.7494±0.0026
	500	0.638	24.5863±0.0020
Ethyl acetate	300	0.382	54.8463±0.0025
	350	0.342	59.5745±0.0015
	400	0.313	63.0024±0.0010
	450	0.275	67.4941±0.0000
	500	0.230	72.8132±0.0006
Hexane	300	0.365	56.8558±0.0026
	350	0.301	64.4208±0.0006
	400	0.209	75.2955±0.0012
	450	0.226	73.2861±0.0012
	500	0.191	77.4232±0.0012

The values of DPPH radical scavenging activity in the table are represented as mean±standard deviation (n=3).

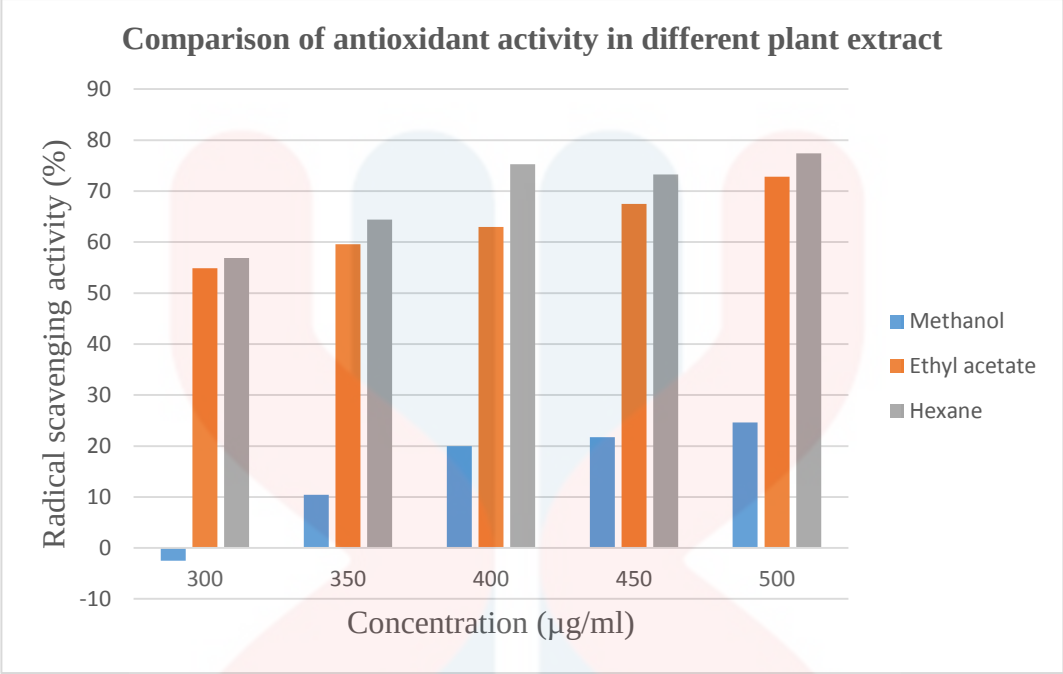

Figure 4.5 The comparison of antioxidant activity in different plant extracts

Figure 4.5 illustrates the comparison of radical scavenging activity of *A. longiloba* Miq. extracts in different solvent with 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 illustrated as 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.

From this study, it presented that the extracts of *A. longiloba* Miq. 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* Miq. showed the lowest radical scavenging activity. This can be supported by (Esmaeili *et al.*, 2015), 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 (Jadid *et al.*, 2017).

Lastly, the hexane extract was the highest antioxidant activity. This statement was supported by (Bae *et al.*, 2012) 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* Miq.

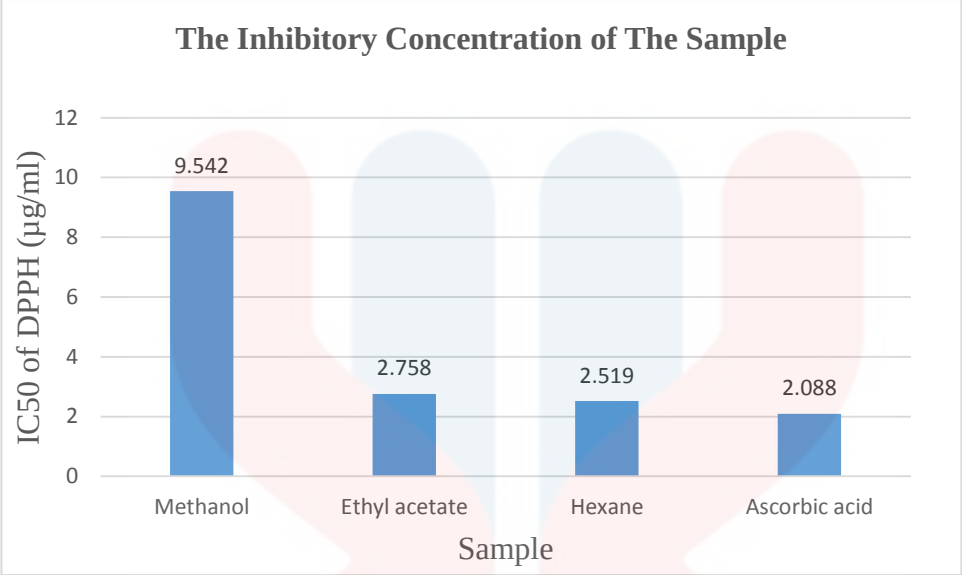

Figure 4.6 The IC50 of DPPH

The antioxidant activity of *A. longiloba Miq*. can be expressed as inhibitory concentration (IC_{50}). The $IC_{50}$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 IC_{50} value from the Figure 4.5 illustrates as descending order which was Methanol> Ethyl acetate> Hexane> Ascorbic acid. Methanol having the highest IC_{50} value with the value of concentration was 9.542 µg/ml which means that methanol extract had the lowest antioxidant activity. Despite of that, hexane extract had the lowest of IC_{50} value among these three extract (2.519µg/ml). The ascorbic acid was used as reference for antioxidant activity and it had the lowest IC_{50} value (2.088 µg/ml) which indicates had the highest antioxidant activity. Hangun-Balkir & McKenney (2012) said that the lower IC_{50} shows the greater effectiveness of the antioxidant.

Jadid *et al*., (2017) 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.

As a conclusion, methanol, ethyl acetate and hexane extract exhibit the potential of antioxidant activity. Nevertheless, methanol extract of *A. longiloba* Miq., showed the weakest antioxidant activity among the other extracts.

CHAPTER 5

CONCLUSION & RECOMMENDATION

5.1 Conclusion

The objectives that have been highlighted in Chapter 1 have been completed by the end of this study. The first objective in this study is to determine the total phenolic content in *Alocasia longiloba* Miq. with different extract solvents. From this objective, it can be concluded that the total phenolic content of *A. longiloba* Miq. in ethyl acetate extract was more efficient as it gave the highest value when compared to methanol and hexane extract. The second objective of this study is to determine the antioxidant activity in *Alocasia longiloba* Miq. by using DPPH assay with different extract solvents. In addition to these objectives, 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* Miq have the potential of antioxidant that can be used for the medicinal purposes.

5.2 Recommendation

This study is focused on the total phenolic content and antioxidant activity in the leaf blades and petioles of *A. longiloba* Miq. in different polarity of extract solvents which were methanol, ethyl acetate, and hexane. In order to improve this study, here are some recommendations which should be considered.

Firstly, as *A. longiloba* Miq., having potential for medicinal purpose, study about the growth development on this plant is very important. The development of new products based on this plant will cause the high demand for this plant. Hence, if this study is run, we can know the average time and development of *A. longiloba* Miq. plant and the harvesting time can be decided.

Next, study on microbial activity on this species is recommended. As there is no information on microbial activity about this plant especially for its leaf blades and petioles. Thus, this study is very useful as it can be used for medicinal purpose.

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APPENDICES

Appendix A: The raw material of experiment

Appendix B: Lab work

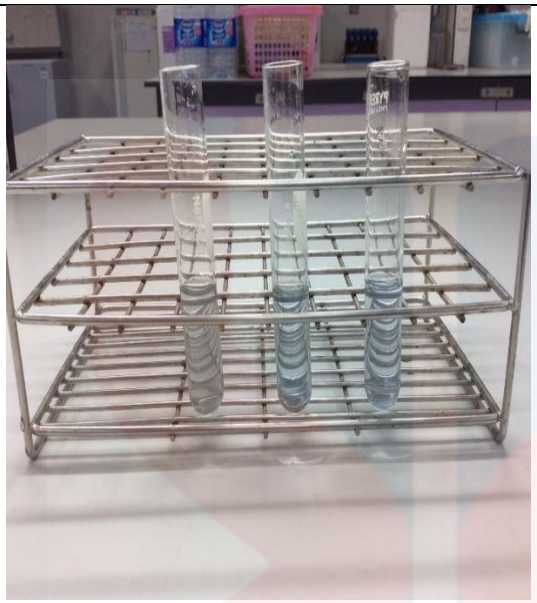
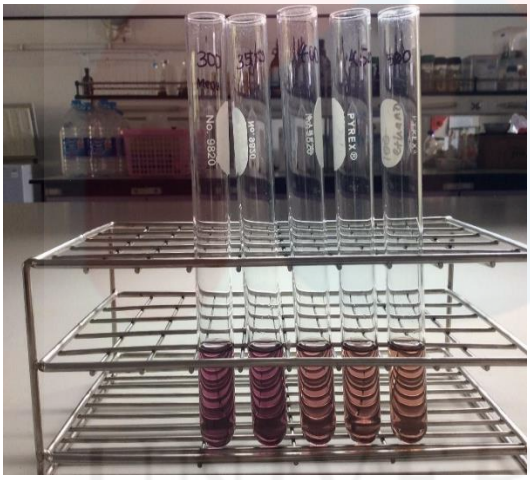
Appendix A

Raw Material	Description
	The *Alocasia longiloba* Miq. plant.

Appendix B

Lab work	Description
	The petioles and blades that have been chopped.
	The dried plant material.
	The fine powder was kept in desiccator.

	Reflux extraction.
	Filtration.

	The total phenolic content of methanol, ethyl acetate and hexane extract.
	DPPH radical scavenging activity of methanol extract.