PHYTOCHEMICAL ANALYSIS OF *Etlingera brevilabrum* (Valeton) R. M. Sm.

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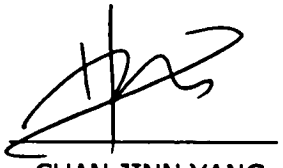

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ABSTRACT

In this study, the effects of red pigmentation coverage on leaf surface of *Etlingera breveilabrum* (Valeton) R. M. Sm. and extraction time on total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (AOA) and antimicrobial activity (AMA) were investigated. The leaves of *E. brevilabrum* were divided into three categories based on the red pigmentation coverage on the leaf surface; L1 (70 – 100%), L2 (35 – 69%) and L3 (0 – 34%). The leaves samples were macerated with methanol solvent and extracted at two different extraction time, E1: 72 hours or E2: 1 hour. The TPC and TFC were estimated using spectrophotometric method. The AOA of extracts was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The AMA of extracts was evaluated using Kirby-Bauer test. The results showed that, there was no interaction between the two factors of red pigmentation coverage on the leaf surface and the extraction time of *E. brevilabrum*. However, the factor of red pigmentation coverage did influence the extraction yield and TFC. The highest extraction yield was observed on L3 (41.33 ± 9.18 %) followed by L2 (34.50 ± 8.76 %) and L1 (33.33 ± 7.89%). For TFC, L1 produced higher TFC (2.48 ± 0.24 mg QE/g) compared to L2 (2.40 ± 0.14 mg QE/g) and L3 (2.27 ± 0.08 mg QE/g). Meanwhile, the factor of extraction time was found to influence all parameters tested. Leaf sample extracted for 72 hours gave higher extraction yield which is 44.33 ± 6.24 %, however, sample that was extracted for 1 hour showed better result for TPC, TFC, and AOA with 47.62 ± 5.14 mg GAE/g, 2.50 ± 0.16 mg QE/g and IC_{50} value of 430.43 ± 55.71 µg/mL respectively. A strong correlation was observed between the TPC and AOA, whereas the TFC showed weak correlation with the AOA. For the antimicrobial activity, it was found that only leaves sample extracted for 1 hour showed inhibition against *Bacillus cereus*. As for recommendations, further research on the effect of extraction time on *E. brevilabrum* leaves can be carried out in order to determine the optimal extraction time of this plant. In addition, further research on the antimicrobial activity of *E. brevilabrum* can be carried out to determine the minimal inhibitory concentration required to inhibit the growth of bacteria.

ANALISIS FITOKIMIA

***Etlingera breveilabrum* (Valeton) R. M. Sm.**

ABSTRAK

Dalam kajian ini, kesan liputan pigmentasi merah pada permukaan daun *Etlingera breveilabrum* (Valeton) R. M. Sm. dan masa pengekstrakan pada jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC), aktiviti antioksida (AOA) dan aktiviti antimikrob (AMA) telah dikajian. Daun *E. brevilabrum* telah dibahagikan kepada tiga kategori berdasarkan liputan pigmentasi merah pada permukaan daun iaitu L1 (70 – 100%), L2 (35 – 69%) and L3 (0 – 34%). Daun tersebut telah melalui two masa pengekstrakan yang berbeza iaitu E1: 72 jam dan E2: 1 jam. TPC dan TFC telah diuji dengan menggunakan cara spektrofotometri. Bagi AOA ekstrak telah diuji dengan menggunakan cara cerakin 1,1-diphenyl-2-picrylhydrazyl (DPPH). Bagi AMA telah diuji dengan menggunakan kaedah Kirby-Bauer. Keputusan kajian ini telah menunjukkan bahawa, tiada interaksi antara dua faktor iaitu liputan pigmentasi merah pada permukaan daun dan masa pengekstrakan *E. breveilabrum*. Walau bagaimanapun, faktor liputan pigmentasi merah telah mempengaruhi jumlah hasil penyarian. Jumlah hasil penyarian yang paling tinggi adalah pada L3 (41.33 ± 9.18 %) diikuti dengan L2 (34.50 ± 8.76 %) dan L1 (33.33 ± 7.89%). Bagi TFC, L1 telah menghasil TFC yang lebih tinggi (2.48 ± 0.24 mg QE/g) berbanding dengan L2 (2.40 ± 0.14 mg QE/g) and L3 (2.27 ± 0.08 mg QE/g). Di samping itu, faktor masa pengekstrakan didapati telah dapat mempengaruhi semua parameter yang telah diuji. Bagi sampel daun yang telah diestrak selama 72 jam telah memberi jumlah hasil ekstrak yang lebih tinggi iaitu 44.33 ± 6.24 %, manakala, bagi sampel yang telah diekstrak selama 1 jam telah memberi keputusan yang lebih baik bagi ke atas TPC, TFC dan AOA berbanding dengan 72 jam pengekstrakan iaitu 47.62 ± 5.14 mg GAE/g, 2.50 ± 0.16 mg QE/g dan nilai IC_{50} 430.43 ± 55.71 µg/mL masing-masing. Satu korelasi yang kuat antara TPC dan AOA telah didapati, manakala, TFC menunjuk korelasi yang lemah dengan AOA. Bagi AMA telah didapati bahawa hanya sampel daun yang diekstrak dengan 1 jam menunjuk halangan kepada pertumbuhan bakteria *Bacillus* cereus. Sebagai cadangan, kajian lanjutan pada kesan masa pengekstrakan daun *E. Brevilabrum* boleh dijalankan supaya dapat menentukan masa optimal pengekstrakan tumbuhan ini. Tambahan pula, kajian lanjutan pada aktiviti antimikrob *E. Brevilabrum* boleh dijalankan untuk menentukan kepekatan rencatan minimum yang diperlukan untuk menhalangkan pertumbuhan bakteria.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percent
°C	Degree Celcius
°	Degree
'	Minute
"	Second
µg/mL	Microgram per milimetre
µL	Microlitre
AMA	Antimicrobial Activity
AOA	Antioxidant Activity
cm	Centimetre
DPPH	2,2-diphenyl-1-picrylhydrazyl
E	East
FCR	Folin-Ciocalteu's Reagent
GAE	Gallic Acid Equivalent
G	Gram
IC_{50}	Inhibitory Concentration
L	Litre
M	Molar
m	Metre
mg/L	Milligram per litre
mL	Milimetre
mg	Miligram
mM	Milimolar
N	North
nm	Nanometre
QE	Quercetin Equivalent
RM	Ringgit Malaysia
RSA	Radical Scavenging Activity
TPC	Total Phenolic Content
TFC	Total Flavonoid Content

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Introduction

It is believed that plants have always been a source of nutrients and immense therapeutic potential (Swargiary and Roy, 2015). Since ancient time, plants such as spices, medicinal herbs, vegetables, fruits, etc., have been used to cure variety of diseases. Today in this modern world, although there are varieties of synthetic drugs which are readily available and highly effective in curing various diseases, however there are still a lot of people who prefer using those traditional folk medicines that produce from medicinal plants instead of synthetic drugs due to their less harmful effects (Iqbal *et al.,* 2015). The medicinal values of those medicinal plants depend on the chemical substances that produce a definite physiological action on the human body (Edeoga *et al.,* 2005). Those important bioactive constituents or secondary metabolites that found in plants are such as alkaloids, tannins, flavonoids and phenolic compounds (Edeoga *et al.,* 2005; Bourgaud *et al.,* 2001). The secondary metabolites that found and isolated from various plant parts which includes the flowers, leave, stem, fruits and roots are found to be beneficial, whereby studies have shown that those compound possess ability such as anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and many other activities to a great or lesser extent (Cai *et al.,* 2004; Miliauskas *et al.,* 2004; Lachumy *et al.,* 2010).

Malaysia has been recognized as a country which has significantly contributed to the herbal industry due to the richness of various biological heritage in medicinal and flowering plant species, an extensive variety of plant species have been recorded to have medicinal value, approximately 7,411 plant species have been identified in Sabah and about 80% of the indigenous plants were used by the local communities (Malaysian Agricultural Research and Development Institute, 2015; Alsarhan *et al.,* 2014). According to the Malaysian Herbal Corporation, the value of herbal industry in

Malaysia is about RM17 billion in the year 2013, and it was predicted that the market value of the herbal industry in Malaysia is projected to reach RM32 billion in the year 2020, with the annual growth rate between 8% - 15%. Thus, the growing market of herbal industry offers an opportunity as wealth creation, especially to to the farmers and young generations.

The plants from the family Zingiberaceae are well-known for its medicinal values and a famous natural resource that used for food, spices, dyes, perfumes and aesthetics all around the world (Mahdavi, 2014). It is widely distributed throughout the tropics area, particularly in Southeast Asia (Kumar *et al.,* 2013). As a largest family of the plant kingdom, Zingiberaceae can be divided into four subfamilies that include Hedychieae, Zingibereae, Alpineae, and Globbeae. Among of these, the genus *Etlingera* belongs to the Alpineae tribe (Mahdavi *et al.,* 2015). *Etlingera* is known as an Indo-Pacific genus which includes more than 100 species that can grows from sea level to an altitude of 2,500m, it is also one of the tallest genus of the family that can grow up to 6m high and is found to distribute from the Himalayas and southwest of China through Burma, Thailand, Malaysia and Indonesia (Mahdavi *et al.,* 2015; Mahdavi *et al.,* 2012).

The plants of *Etlingera* consist of various commercial and traditional uses. For example, the inner part of the young stems, flower buds and fruits of *Etlingera elatior*, *Etlingera littoralis* and *Etlingera rubrolutea* are consumed by the indigenous communities in Sabah, Malaysia as spices, eaten raw or cooked (Mahdavi *et al.,* 2013b). Besides, the plants of *Etlingera* are also known to have some medicinal uses.

1.2 Justification

In this study, *Etlingera brevilabrum* (Valeton) R. M. Sm. was chosen as a studied plant. *Etlingera brevilabrum* is known to be endemic to Borneo, and was used as a medicinal plant for the local people to combat against long-lasting fever in children by rubbing the body with the roasted leaves, the sap from the stems can be used to treat eye problems, and the base can be used as a medicine to relieve stomach pain (Mahdavi *et al.,* 2013b). *E. brevilabrum* plant is easily to be identified by the red pigmentation on its leaf surface. However, the red pigmentation coverage was not same to the other leaves in the same plant, thus make it hard to select which leaf can be used for medicinal purpose. Therefore, this study was conducted to see the effect of red

pigmentation coverage on the leaves surface of *E. brevilabrum* on the total phenolic content, total flavonoid content, antioxidant activities and antimicrobial activities. The finding from this study will benefit to future research during sample collection of this particular plant.

1.3 Objective

i. The objective of this research is to determine the effect of red pigmentation coverage on the leaves of *Etlingera brevilabrum* on the total phenolic, flavonoid content, antioxidant activity and antimicrobial activity.

ii. The second objective of this research is to determine the effect of different extraction time on the total phenolic, flavonoid content, antioxidant activity and antimicrobial activity of *Etlingera brevilabrum*.

1.4 Hypothesis

H_{O1}: There is no significant difference on the effect of red pigmentation coverage on the leaves of *Etlingera brevilabrum* on the total phenolic, flavonoid content, antioxidant activity and antimicrobial activity.

H_{A1}: There is significant difference on the effect of red pigmentation coverage on the leaves of *Etlingera brevilabrum* on the total phenolic, flavonoid content, antioxidant activity and antimicrobial activity.

H_{O2}: There is no significant difference on the effect of different extraction time on the total phenolic, flavonoid content, antioxidant activity and antimicrobial activity of *Etlingera brevilabrum*.

H_{A2}: There is significant difference on the effect of different extraction time on the total phenolic, flavonoid content, antioxidant activity and antimicrobial activity of *Etlingera brevilabrum*.

CHAPTER 2

LITERARTURE REVIEW

2.1 *Etlingera*

Etlingera is an Indo-Pacific genus with more than 100 species that growing from the sea level to 2500m. It is native to Bangladesh, Burma, Brunei, China, Cambodia, India, Indonesia, Laos, Malaysia, Philippines, Singapore, Thailand, Vietnam, Papua New Guinea, Australia and several Pacific islands. Most of the species are found near to the equator. Among of the area stated above, it was found that Borneo is one of the area that to have the highest species richness (Axel, 2006).

The genus *Etlingera* is monophyletic, with the genus *Hornstedtia* as its sister group. The unique character diagnostic of all species of the genus is a tube formed above the insertion of the corolla lobes (Axel, 2006).

Etlingera consists of terrestrial and perennial herbs. Some of the larger species have leafy shoots that can attain nearly 10 m. the base of these shoots become very stout and do not appear very herb-like. Some species have the leafy shoots in a clump; others have long-creeping rhizomes, making the leafy shoots growing more than one metre apart.

2.2 *Etlingera* in Borneo

There are several species of *Etlingera* that occur in Borneo were first described from the Malay Peninsula. Of the 40 species of *Etlingera* recognised in the present account, 80% are found in Sarawak, 70% in Sabah, 33% in Brunei and 65% in Kalimantan. Smaller species-rich areas include Gunung Kinabalu in Sabah which harbours 16

species, 40% of the species known from Borneo. Gunung Mulu in Sarawak has a similar number.

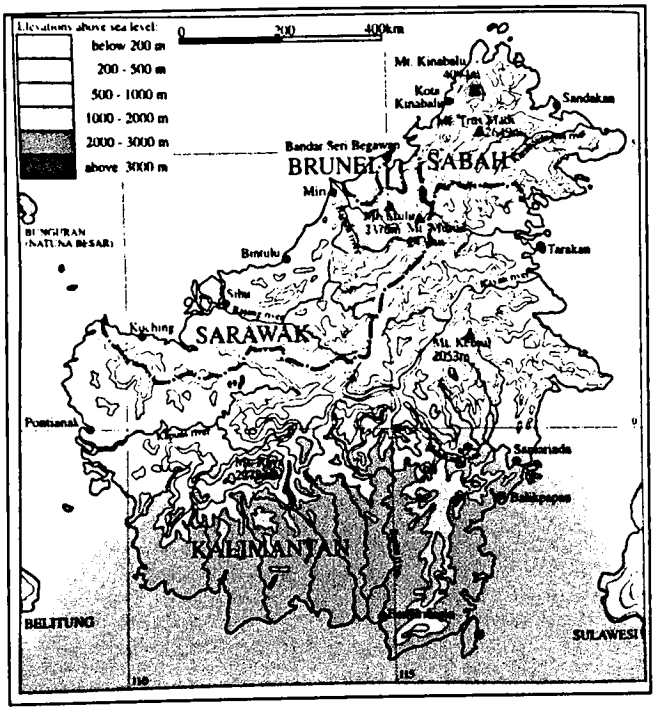

Figure 2.1: Borneo has a surface area of 740,000 km^2 and is shared by three nations: Malaysia (the states of Sabah and Sarawak), Brunei Darussalam and Indonesia (Kalimantan).

Source: Axel, 2006. *Etlingera* of Borneo, Natural History Publications (Borneo).

2.3 *Etlingera brevilabrum* (Valeton) R. M. Sm.

Etlingera brevilabrum is the genus of Indo-Pacific perennial herbs and terrestrial of a ginger family of Zingiberaceae, which is endemic to Borneo. *E. brevilabrum* has a variety of traditional used by the local people. For examples, the fruits are edible by the local people and animals, leaves used medicinally for children against long-lasting fever by rubbing the body with the roasted leaves; applied to diseased skin that looks dry, on legs only, base used as medicine against stomach-ache and young shoot was heated and the juice was squeezed and used as eye drops to treat sore eyes. The plants usually grow in deep shade in dense primary forest or in marshy, young or old secondary or logged mixed dipterocarp forests, swidden fallows, and old gardens, on hills, on slope, flat areas or near to riverbanks and in limestone, sandstone, sandy or rich clay soils at the elevation range from 9 to 1140 m (Axel, 2006).

E. brevilabrum is an erect plant that comprises of long creeping rhizome which shoots up to 1 m or more apart and leafy shoot which can grow 1.5 – 5 m with up to 10 – 18 leaves but often found leafless at the base. The leaves are found to be in oblong obovate shape, smooth, green with reddish cloudy patches on the surface and red at the base or greenish; the lowest sheath sometimes pubescent. The flowers are found to be grown at the base of the plant that in shiny reddish pink colour (Figure 2.3). The fruit of *E. brevilabrum* come with a size of 2.5 × 3.5 cm, which is in golden brown colour, round shape and only slightly longitudinally ridges or warty on the sides.(Axel, 2006).

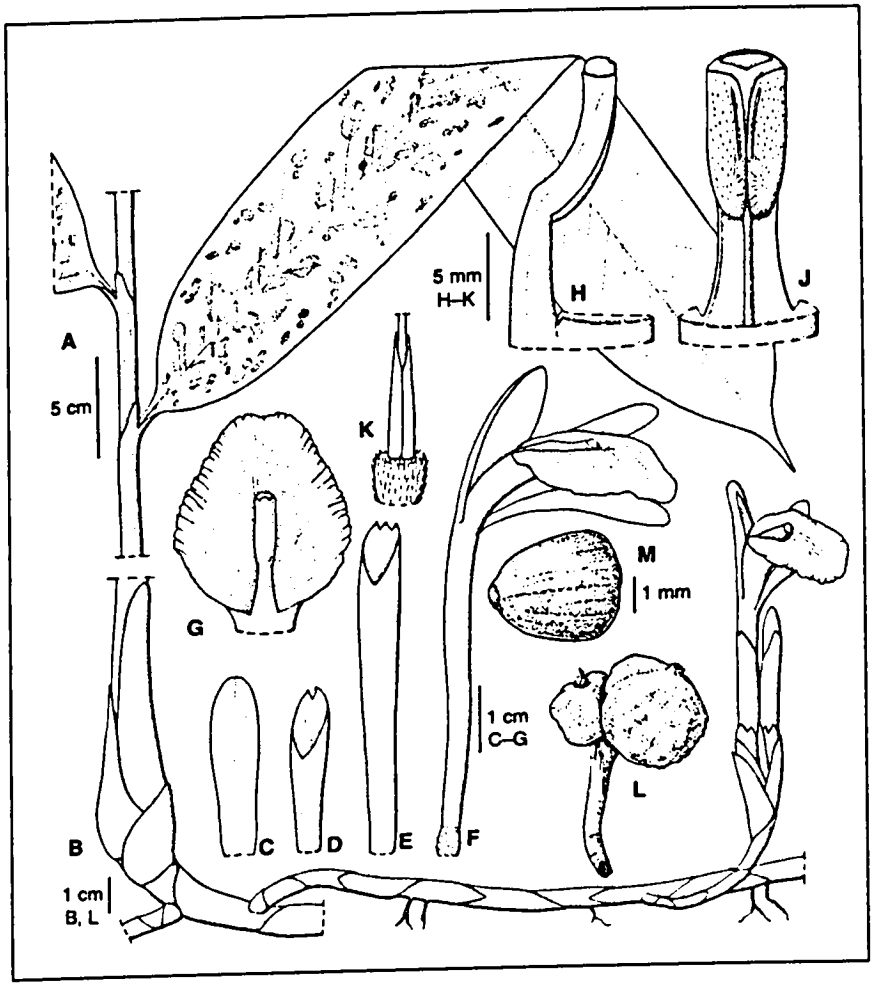

Figure 2.2: Morphology of *Etlingera* brevilabrum (Valeton) R. M. SM. **A.** Leaf. **B.** Base of leafy shoot with rhizome and inflorescence. **C.** Fertile bract. **D.** Bracteole. **E.** Calyx. **F.** Corolla. **G.** Stamen and labellum, dorsal view. **H.** Stamen, lateral view. **J.** Stamen, ventral view. **K.** Epigynous gland on top of ovary. **L.** Infructescence. **M.** Seed, aril removed.

Soruce: Axel, 2006. *Etlingera* of Borneo, Natural History Publications (Borneo).

Figure 2.3: Flowers of *Etlingera brevilabrum* (Valeton) R. M. SM.

Source: Axel, 2006. *Etlingera* of Borneo, Natural History Publications (Borneo).

Figure 2.4: Fruits of *Etlingera brevilabrum* (Valeton) R. M. SM.

Source: Axel, 2006. *Etlingera* of Borneo, Natural History Publications (Borneo).

2.4 Phytochemical Constituents in Plant

Phytochemicals in plants also known as bioactive compounds are compounds produced by the plants which having pharmacological or toxicological effects in humans or animals. Although nutrients elicit pharmacological or toxicological effects when ingested at high dosages, nutrients in plants are generally not included in the term bioactive plant compound. The typical bioactive compounds in plants are produced as secondary metabolites. The examples of secondary metabolites that can be found in plants are glycosides, saponins, flavonoids, tannins, terpenoids, resins, lignans and alkaloids (Bernhof, 2010).

The secondary metabolites are produced within the plants besides the primary biosynthetic and metabolic routes of compounds aimed at plant growth and development, such as carbohydrates, amino acids, proteins and lipids. The secondary metabolites can be regarded as the products of biochemical "side tracks" in the plant cells and does not needed for plants in their daily function. Although secondary bioactive compounds in plants appear to be randomly synthesised, however several of them found to hold the important functions in the living plants (Bernhof, 2010).

For instance, flavonoids help to protect the plants from free radicals generated during photosynthesis. Terpenoids function as to attract pollinators or seed dispersers, or inhibit competing plants. Alkaloids usually help to protect the plants from the attacking of herbivore animals or insects. While some of other secondary metabolites function as cellular signalling molecules or other functions in the plants (Aksel, 2010).

As general, plants with potent bioactive compounds are often being characterised as both medicinal and poisonous, and a beneficial or an adverse result may depend on the amount of intake (Bernhof, 2010).

2.4.1 Phytochemicals in *Etlingera* spp.

There are still limited of studies conducted on the phytochemicals contents in *Etlingera brevilabrum*. However there are several studies on the phytochemicals screening on the other *Etlingera* genus plants.

It was found that the phytochemicals that available in the flower extract of plant *Etlingera elatior* (torch ginger) showed the prescence of flavonoids, terpenoids,

saponins, tannins, and carbohydrates (Lachumy *et al.*, 2010), phytochemicals such as cardiac glycosides and steroids were presence in all plant parts of *Etlingera coccinea*, while saponins and anthraquinones were presence in the leaves and stems (Shahid *et al.*, 2015) and phytochemicals such as alkaloids, glycosides, steroids, tannins, flavonoids, saponins and reducing sugars were presence in the extract of *Etlingera linguiformis* (Arafat *et al.*, 2013).

The presence of these phytochemicals has been contributed for it medicinal value. For example, the presence of saponins has abundant of medicinal use as expectrorants and treatment for excessive salivation, chlorosis and migraines. While the presence of tannins also contributed to a wide variety of usage such as antibacterial, antiviral, anti-inflammatory and antiparasitic. Both flavonoids and tannins are phenolic compounds that act as primary antioxidants or free radicals scavengers. Plants steroids have the potential to function as effective, natural and safe alternatives to treat age and disease-associated muscle loss or to improve endurance and physical performance (Lachumy *et al.*, 2010; Shahid *et al.*, 2015).

As general, through the previous studies of those *Etlingera* genus plants, it is believed that the phytochemicals that presence in those *Etlingera* genus plants might also be presence in the plant *E. brevilabrum* since they are the same genus and family of zingiberaceae.

a. Total Phenolic Content

There are three major classes of plant chemicals; phenolic metabolites, terpenoids and alkaloids. Among of these groups, phenolic compounds are found to be the most important dietary applications (Do *et al.*, 2014). Phenolic compounds are the secondary metabolites. They present in a large diversity of structures, including rather simple molecules (e.g. gallic acid and caffeic acid), polyphenols (e.g. hydrolyzable and tannins), phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and flavonoids. (Cheynier, 2012; Do *et al.*, 2014).

Many plants are known to have phenolic compounds that exhibiting the antioxidant activity and proposed for protection against oxidation (Ghasemzadeh *et al.*, 2010; Maisuthisakul *et al.*, 2005). Extracts from plants were found to contribute health benefits to consumers, arising from protection from free radical-mediated

deteriorations, and which cause retardation of lipid oxidation that had stronger antioxidant activity than those synthetic antioxidants (Maisuthisakul *et al.,* 2005). Furthermore, the use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants were found to have genotoxic effect (Stankovic, 2010).

b. Total Flavonoid Content

Flavonoids are a large family of over 4000 secondary plant metabolites that possess a wide range of biochemical and pharmacological effects which including anti-oxidation, anti-inflammation, anti-platelet, anti-thrombotic action, and anti-allergic effects (Miean *et al.,* 2001). Flavonoids help to inhibit enzymes such as prostaglandin synthase, lypoxygenase, and cyclooxygenase that are closely related to tumorigenesis and induce detoxifying enzyme systems such as glutathione S-transferase. (Miean *et al.,* 2001).

Flavonoids are polyphenols with the diphenylpropanes ($C_6C_3C_6$) skeletons structure. There are four major classes of flavonoids which are the 4-oxo-flavonoids (e.g. flavones, flavonols, etc.), anthocyanins, isoflavones, and the flavan-3-ol derivatives (Miean *et al,* 2001). Furthermore, flavonoids are found to have the function to reduce blood-lipid and glucose and to enhance human immunity. In some previous studies suggested that flavonoids such as catechin and quercetin could be able to control cancer cell growth in the human body (Ghasemzadeh *et al.,* 2010a).

There is still limited of studies on the total flavonoid content in *Etlingera brevilabrum*. However, according to some previous studies on the total flavonoid content in some zingiberaceae plants, flavonoids such as kaempferol 3-glucuronide, quercetin 3-glucuronide, quercetin 3-glucoside, and quercetin 3-rhamnoside were found to present in the leaf of *Etlingera elatior* (Williams *et al.*, 1977; Chan *et al.*, 2007a). Furthermore, previous study also reported that the leaves of *Etlingera coccinea* also consists of higher total flavonoid content than those of stems and rhizomes (Shahid *et al.,* 2015). Meanwhile, studies also reported that, flavonoids were found to be present in the leaves of gingko, lotus, and ginger (Feng *et al.,* 2002; Chen *et al.,* 2002; Ghasemzadeh *et al.,* 2010b).

2.5 Antioxidant Activity in *Etlingera* spp.

Free radicals were found to contribute more than one hundred types of disorders in the human including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Pourmorad *et al.,* 2006). Besides, free radicals due to the environmental pollutants, radiation, chemicals, toxins, physical stress can cause depletion of immune systems antioxidants, change in gene expression and induce abnormal proteins (Pourmorad *et al.,* 2006). Due to the depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be needed (Pourmorad *et al.,* 2006).

It is believed that medicinal plants possess therapeutic potentials as antioxidants in reducing free radical. The antioxidant activity of the plants might be due to the present of phenolic and flavonoid compounds that had the properties of free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Pourmorad *et al.,* 2006). One of the rapid and sensitive method to screen the antioxidant activity of plant extracts is through free radical scavenging assay by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) (Pourmorad *et al.,* 2006).

According to previous study on the antioxidant activity of *Etlingera brevilabrum* was carried out to evaluate the activity in different parts of the plant using the DPPH scavenging activity method. Among the results, leaf extracts showed the highest antioxidant activity with the lowest concentration to reach the IC_{50} than the other parts (Mahdavi *et al.,* 2013a). Meanwhile, in the study of Chan *et al.* (2007a), antioxidant activity of leaves of *Etlingera* species also being tested by the DPPH free radical scavenging method as well as in the study of Sabli *et al.* (2012), antioxidant activity of selected *Etlingera* and *Zingiber* species.

2.6 Antimicrobial Activity in *Etlingera* spp.

The use of alternative medical therapy has increased the interest of pharmacologists and herbalists over the past decade. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. A large number of plants were used to combat different diseases and known to possess antimicrobial activity (El *et al.*, 2005). Several studies have been revealed that antimicrobial activities in plants are due to the present of

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