TOTAL POLYPHENOL CONTENT, TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF *ETLINGERA ELATIOR* FLOWER

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DISSERTATION SUBMITTED AS PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

INDUSTRIAL CHEMISTRY PROGRAMME
FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH

2014

222314

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: TOTAL POLYPHENOL, TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL OF ETLINGERA ~~EL~~ ELATIOR

IJAZAH: SARJANA MUDA DENGAN KEPUJIAN KIMIA INDUSTRI

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VALIDATION

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ACKNOWLEDGEMENT

Foremost, Alhamdulillah, I would like to thank to ALLAH S.W.T for blessing me with health and faithfulness to finish up my final year project. I would also like to express my sincere gratitude to my supervisor Dr. Mohammad Shaheen Khan for his guidance in helped me in all the time of research and writing of this thesis. Besides my supervisor. This thesis would have never been accomplished without their assistance, supports and dedicated involvement throughout the process. Not forget to all lecturers that involved in teaching the industrial chemistry course

My sincere thanks also goes to my beloved family, my parents, sisters and brothers for giving me encouragement and the continuous support throughout this project. I would also like to show gratitude to my friends, my fellow classmate and lab partner for their help, understanding and supports throughout this final year project

ABSTRACT

In this study, the total polyphenol, flavonoid and antioxidant activity of essential oil of *Etlingera elatior* were investigated. The essential oil of *E. elatior flower* was extracted by using hydrodistillation and the chemical composition were analysed by using GC-MS. The major component of the essential oil of *E. elatior* flower were 1-dodecanol (60.80 %), dodecanal, and 1,1-dodecanediol, diacetate with 32.27 %, methyl-cyclopentane (16.39 %), heptane (5.92 %), lauryl acetate (5.91 %), cyclotetradecane (5.05%) and α-pinene (2.32 %) with the percentage yield of essential oil of 0.031 %. The total polyphenol were determined by using Folin-Ciocaltue method and external calibration with Gallic acid while the total flavonoid content were determined by using aluminium chloride colorimetric method. The antioxidant activity of essential oil of *E. elatior* flower were measured by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay by measured its percentage of inhibition activity reduced by 50 % of DPPH (IC_{50} %).The total polyphenol content of essential oil of *E. elatior* flower is 73.1± 0.0001 mg GAE/g while the total flavonoid content is 190.200 ± 0.0003 mg QE/g. The antioxidant activity of essential oil of *E. elatior* flower is low than synthetic antioxidant of butylated hydroxytouluene (BHT) with the value of 0.03 mg/mL of IC_{50}, while the IC_{50} value of BHT is 0.00446 mg/mL. Thus it is clearly shows that the antioxidant activity of essential oil of *E. elatior* flower is low, since the higher the antioxidant activity, the lower the value of IC_{50}.

ABSTRAK

Dalam kajian ini, jumlah polifenol, flavonoid dan antioksida daripada minyak pati Etlingera elatior dikaji. Minyak pati daripada bunga E. elatior diekstrak menggunakan penyulingan hidro dan komposisi kimia di kaji menggunakan kromatografi gas spektrometri jisim (GC-MS). Komponen-komponen utama yang dikenal pasti di dalam miyak pati daripada bunga E. elatior ialah 1-dodecanol (60.80 %), dodecanal, dan 1,1-dodecanediol, diacetate (32.27 %), methyl-cyclopentane (16.39 %), heptane (5.92 %), lauryl acetate (5.91 %), cyclotetradecane (5.05%) dan α-pinene (2.32 %) dengan peratusan hasil sebanyak 0.031 %. Jumlah kandungan polifenol dikaji menggunakan kaedah Folin-Ciocaltue dan penentukuran luar daripada asid galik manakala jumlah kandungan flavonoid dikaji menggunakan kaedah kolorimetrik daripada aluminium klorida. Aktiviti antioksidan daripada minyak pati daripada bunga E. elatior dikaji menggunakan kaedah daripada 1, 1-diphenyl-2-picrylhydrazyl (DPPH) dengan mengira peratusan aktiviti perencatan yang dikurangkan sebanyak 50 % oleh DPPH (IC_{50}). Jumlah kandungan polifenol daripada minyak pati daripada bunga E. elatior ialah 73.1± 0.0001 mg GAE/g manakala jumlah kandungan flavonoid ialah 190.200 ± 0.0003 mg QE/g. Aktiviti antioksidan daripada minyak pati daripada bunga E. elatior adalah rendah berbanding antioksidan sintetik iaitu butylated hydroxytouluene (BHT) dengan jumlah sebanyak 0.03 mg/mL (IC_{50}) manakala jumlah (IC_{50}) untuk BHT ialah 0.00446 mg/mL.Oleh itu, ini jelas menunjukkan bahawa aktiviti antioksidan bagi minyak pati daripada bunga E. elatior adalah rendah, ini disebabkan jika aktiviti antioksidan tinggi maka nilai bagi (IC_{50}) ialah rendah.

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Etlingera elatior	*E. elatior*
Gas chromatography mass spectrometer	GC-MS
1,1-diphenyl-2-picrylhydrazyl radical	DPPH
Aluminium chloride	$AlCl_3$.
Milligrams of the total gallic acid over gram	GAE mg/g
Milligrams of the total quercetin over gram	mg QE/g
Gram	g
Milligram	mg
Millilitre	mL
Total polyphenol content	TPC
Total flavonoid content	TFC
Radical scavenging activity	RSA
Butylated hydroxytoulene	BHT
Inhibition concentration reduced by 50 %	IC_{50}

CHAPTER 1

INTRODUCTION

1.1 Background study

About 80% of the people that living in the developing countries depend almost on traditional medicine that has been estimated by the World Health Organization (Trivedi *et al.*, 2006). The herbal medicine is also named as the botanical medicine or phytomedicine. Essential oils are bioactive compounds which has been used for long time ago in food preservation, pharmaceuticals, traditional medicine and natural therapies (Imelouane *et al.,* 2009). Since the ancient times, essential oils are known for their medicinal advantages as well as their powerful compounds to heal both body and mind (Abdelouaheb and Amadou, 2010). In addition, for thousands of years, the essential oil of aromatic plants are reported to have rich sources of secondary chemical products and their derivatives. Thus, the essential oils that relies predominantly on plants gives the advantageous in health care and also clinically sources of useful drugs. (Mohamed *et al.,* 2013). In the era of the Renaissance, Europeans have already undergo the further research of essential oil with the development of the composition and nature of essential oils have been also created and investigated (Abdelouaheb and Dicko, 2010).

Earlier study shows that approximately there are 3000 essential oils are known, 300 of them are identified important to pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries (Bakkali *et al.,* 2008). Essential oil that extracted from the herbal medicinal plant has a potential to treat some of diseases which become progressively important for human health. The earliest times mankind has been using medicinal plants in order to cure a disease and reduce physical pain (Pradeep *et al.,* 2003). Essential oils play an important role in nature in order to act as antibacterials, antivirals, antifungals, insecticides and also against their herbivores by reducing their appetite for such plants. Besides they also may attract some of the insects to favour the dispersion of pollens and seeds or repel the others that disadvantageous (Bakkali *et al.,* 2008)

The medicinal plants consist of higher amount of polyphenol compounds than the vegetables and fruits. Besides the most polyphenol compound that has been identified in these medicinal plants are flavonoid, tannins, coumarins, lignin, quinones, stilbenes and curcuminoids (Nizar *et al.,* 2013). Phenols play an important property of adsorbing and neutralizing free radicals that has a potential function as antioxidant, anticancer, antiviral, antibacterial, and anti-inflammatory activities (Prakash *et al.,* 2007). Besides, flavonoid also one of the natural major component that are found in the plants that brings to positive effect to human health. There are more than 4000 flavonoid has been known. Some of the flavonoids has the important uses of anti-allergic, antitumor, and anti-inflammatory activities (Gil *et al.,* 1994). Thus, it is clearly shows that medicinal plants have many advantages which play an important role to our life.

Natural antioxidant can be widely found from many herbal plants in Malaysia. The tropical climate and the structure of the land support the growth of herbal plants. These herbal plant have being used for medical purpose or consumed as fresh vegetables. The herbs are believed to have a high amount of antioxidant activities. *E. elatior* contain high quantity of phenolic compound which is the sources of the natural

antioxidant property (Wijekoon *et al.*, 2010). According to the Yan and Asmah (2010), torch ginger flower and tumeric leave have high amount phenolic contents, antioxidant and scavenging activities. Essential oils are also extracted from the aromatic plants by the extraction process. Aromatic plants that consist of significant fragrances and tastes has significant amount of essentials oils. Almost 3000 essentials oils are recognized in which commercially important particularly In pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. In addition, essential oils also has been used in massage but specially used in aromatherapy (Bakkali, 2008). Essential oils composed of heterogeneous group of complex mixtures of organic substances (Bettaieb *et al.,* 2010). Aromatic plants can be found all over the world in temperate, subtropical and tropical regions of the earth. Nevertheless, only around one percent of the plant species on the planet are aromatic (Jennifer *et al.,* 2007).

Essential oils are made up of diverse types of terpenoids and volatile organic compounds and shows antimicrobial, antifungal and anti-bacterial properties (Singh *et al.*, 2010). The early method in extracting the essentials oils is including steeping the plants in water, oil or fatty materials. In the modern technology nowadays, the essential oils can be extracted by both chemical and physical methods. There were many method that has been applied to assess and unearth new antioxidant, antimicrobial and antifungal properties from various kind of natural resources such as soil, microorganisms, animals and plants (al-Nomaani *et al.*, 2013).One of the medicinal plants at Sabah was chosen is torch ginger flower. The scientific name of this plant is *E. elatior* flower. This plant also known as Pink Ginger Bud, Pink Torch Ginger, Torch Ginger, Ginger Flower, and Red Ginger in England. In China this plants known as Xiang Boa Jiang and in Malaysia, this plant called as 'bunga kantan'. *E. elatior* flower is one of the medicinal plants that rich of essentials oil. *E. elatior* flower is belong to the family of *Zingiberaceae* which is the member of ginger. *E. elatior* flower is a prevalent plant in South-East Asia which used traditionally for cooking and medicinal purposes (Chan *et al.,* 2011).The centre of diversity for *Zingiberaceae* is the region of Indo Malayan. There approximately 20 genera and 300 species found in Malaysia out of 50 genera 1500 species around the world (Faridahanim *et al.,* 2007). The family of *Zingiberaceae* was the largest plant kingdom (Puang, 1999). The family of the ginger

consist of 1200 species, 1000 of the species are found in tropical Asia. *Zingiberaceae* divided into four major classes which were *Hedychieae, Zingibereae, Alphaneae* and *Globbeae.* The genus of *E. elatior* flower is under the *alphaneae* classes. The genus of the *E. elatior* flower is the one of the genus that produce colourful fruits and flowers (Larsen *et al.*, 1999).

1.2 Objective of Study

The objective of the study:

i. To extract the essential oil from *E. elatior* flower by using hydro-distillation process.

ii. To analyse the chemical composition of essential oil of *E. elatior* flower by gas chromatography-mass spectrometry (GC-MS).

iii. To determine the total flavonoid content and total polyphenol content of essentials oil of *E. elatior* flower and to determine the antioxidant activity.

1.3 Scope of Study

The sample of Sabah medicinal plant used is *E. eliator* flower, the essential oil extracted by using hydro-distillation method. The chemical composition of essentials oil of *E. elatior* flower analysed by using GC-MS. Total flavonoid content of the essential oil of *E. eliator* flower determined by using aluminium chloride colorimetric method while the total polyphenol content of essential oil of *E. eliator* flower determined by using the Folin-Ciocalteu method. Antioxidant activity of essential oil of *E. eliator* flower was determined by using in-vitro free radical scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) test.

CHAPTER 2

LITERATURE REVIEW

2.1 *Etingera Elatior* (Torch Ginger Flower)

E. elatior flower is the genus of Indo-Pacific perennial herbs and terrestrial of a ginger family of *Zingiberaceaef* that comprises of more than 100 different local species which can be found at Indonesia, Vietnam, Thailand and Malaysia and extensively cultivated and neutralized in South East Asia (Lachumy *et al.*, 2010). The *Zingiberaceae* is a tropical monocotyledonous family. Many of the species belong to this family are able to produce essential oils, mainly in their seeds and rhizomes (Donald *et al.*, 2009). Usually, the plant called as 'torch ginger' and it is one of 15 species that has been recognized in Malaysia (Lim, 2001). This plant popurlarly known at Malaysia as "bunga kantan". In addition, this plant is considered to be a native of Sumatra, Indonesia and it is known as "kecombrang" or as "kincung" (Wijekoon *et al.*, 2011). The genus of the plant is named after the German botanist Andreas Ernest Etlinger while the name of *eliator* is the Latin word that has a meaning of "taller" (Wong, 2008) . Torch ginger flower is widely used for traditional purposes, the young shoots, flower buds, or fruits are consumed local communities raw as condiment or cooked. Besides, this plant very popular used as spice for food flavouring (Wijekoon *et al.*, 2011). In addition, this plant has been used in traditional food for Malaysian such as 'asam laksa', ' nasi kerabu' and 'nasi ulam' (Chan *et al.*, 2011). Moreover, *E. elatior* flower can reduces the odour of fish, as well as inhibits pathogenic bacteria and moulds on food (Naufalin, 2005).

Table 2.1 shows the summarized scientific classification of *E. elatior* flower(torch ginger flower) (Wichman, 2005), whereas the picture of the *E. eliator* shown in Photo 2.1 and photo 2.2.

Photo 2.1 *E. elatior* flower

Table 2.1 Scientific classification of *E. eliator* flower (Sources: Wichman, 2005)

Kingdom	Plantae-Plants
Subkingdom	Tracheobionta-Vascular plants
Superdivision	Spermatophyta-Flowering plants
Division	Magnoliophyta-Flowering plants
Class	Liliosida-Monocotyledons
Subclass	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae-Ginger family
Genus	Etlingera Giseke-Wax flower
Species	Etlingera eliator (Jack) R.M. Sm-torch Ginger (National Plant Databases, 2004)

2.1.1 Morphology and Distribution of *E. elatior* flower

E. elatior flower is rhizomatous in nature that grow on large-growing herbaceous plant. The torch ginger flower grows well at the well-drained and moisture soil. This plant has a green and red leaves that grows to a height of about 15 feet with the large red or pink flowers which grow on separate stalks. The flowering season will be on spring and summer. The shoot of leaves can reach the height of about 3 meters with a diameter of about 4 cm. The length of strap-like leaves can grow up for about 80 cm (Wilson, 2008).

Photo 2.2 *E. elatior* flower

The flower of the *E. elatior* flower grows in interesting way, by arising from the rhizome beneath of ground and it is protected with a series of bracts. It is supported by on the scape which can reach a height of more than 60 cm long. The thickness diameter of the scape is about 1 to 2.5 cm long. The external protective gradually open when the maximum height is achieved. Thus, these outer bracts become reflexed to form a 'rim' of the burning torch, then exposes a central with pinecone-like structure that comprising of small tight bracts that form the 'flame'. The true flowers will arise between the bracts found on the pinecone-like structure (Wilson, 2008).

Ginger is one of the family members of Zingiberaceae which is in the same family as the *E. eliator*, the recent study shows that ginger is the valuable traditional medicine which is act as antioxidant and pharmacological agents. The ginger essential oil is the mixture of monoterpenic and sesquiterpenic compounds which include zingiberene, β-bisbolene, cadinene, β-sesquiphellandrene, neral and geranial (Raghavan, 2007). The mixture of ginger oleoresin composed of gingerols and shogaols, among which [6]-gingerol is a major pungent compound. In a study conducted by Qian and Liu, 1992, ginger also exhibit anti-cholinergic and antihistaminic actions and produces anti-motion sickness action possibly by central and peripheral anti-cholinergic and antihistaminic effects.

Moreover, the past research has shown that the *E. elatior* flower was not exhibit any antifungal activity since the previous study, Adams has reported that antifungal activity of essential oil is controlled to a greater extent by oxygenated terpenes, this is due to the limited hydrogen capacity and low water solubility of hydrocarbons that makes they tend to be a relatively inactive compound (Jeevani *et al.,* 2011). Thus, this might be the main causes for torch ginger flower not to exhibit any antifungal activity. In addition, *E. elatior* flower also has been known because its medical properties among indigenous communities in Malaysia.

Decoction that has been prepared from the fruit of *E. elatior* flower are used to treat ear ache and the leaves can be used in healing a wound. The young flower shoot of *E. elatior* flower was reported to have important properties of antitumor, antimicrobial, and cytotoxic (Habsah *et al.*, 2005). Moreover, the phytochemical studies on *E. elatior flower* rhizomes led to separation of two new and six known compounds of diarylheptanoids, labdane diterpenoids, and steroids (Habsah *et al.,* 2005).

2.2 Essential oil

2.2.1 Definition and historical overview of Essential oil

Essential oil of plants are lipid-soluble mixtures of volatile constituent that are found in different parts of aromatic plants like flowers, leaves, seeds and the other plant organ. Essential oils are concentrated volatile liquid which is non-water-soluble aroma compounds that extracted from plants. They are usually obtained by steam or hydrodistillation first developed in the middle Ages by Arabs. Essential oils identified as aromatic liquid that has own essence and are formed as natural mixtures of volatile and complex organic compound. Besides, essential oils also defined as concentrated hydrophobic liquid which comprises of volatile compounds from the plant and expresses its own aroma (Valgimigli, 2012).

Variety of essential oils have been commercially used in ritually, socially and medicinally at different historical view. The history of development and uses of essential oils run correspondingly with human development of civilization in some respect at five or six thousand years ago (Butler, 2000). Essential oils play an important role in nature which is protect the plants as antibacterials, antivirals, anti-fungals, insecticides and also against herbivores by reducing their appetite for such plants. Moreover, they also may attract some of insects to choose the dispersion of pollens and seeds, or repel undesirable others (Bakkali *et al.,* 2008). Essential oils are termed after physical process of aromatic plants in a single type and the odour it has (Valgimigli, 2012).

Essential oils are made up of mixture from the complex monoterpenes group. Monoterpenes is a secondary metabolites which is a phytochemical and it does not have clear function in primer plants metabolism. The chemical composition of essential oils is different and unique between each species (Daferera *et al.*, 2003). Commonly, essential oils is found in specific parts of aromatic plants such as in the petal, leaves, wood, fruits, seeds, roots, rhizomes, resin, gums and also found in more than one part of plant or found from specialized cells (Burt, 2004). All the *Etlingera* species contained

of essential oil. Thus, *Etlingera* species is frequently used in the industry and have high demand for its fragrances and flavour because of high content of essential oils. The past research shows that the highest amount of the essential oil usually present in the rhizome of the *Etlingera* species (Jaafar *et al.,* 2007).

2.2.2 Chemistry and Structural Composition of Essential Oils

Essential oils is a mixture of volatile lipid-soluble organic molecules that has heterogeneous structure. Essential oils are composed of several classes of chemical constituents of hydrogen, oxygen, carbon and a few of nitrogen and sulphur. The physical properties of essential oils is low molecular mass which is normally has less than 400 amu and it has a low polarity. These two types of properties are responsible for their distinctive physical-chemical properties of good volatility and insoluble in water (Valgimigli, 2012). Essential oils are extracted from diverse of aromatic plants which generally localized in temperate countries such as Mediterranean and tropical countries (Bakkali *et al.,* 2008).

One type of oil may comprise of as many as 75 individual constituent as the case of oil of *camphor wood* or also may contain largely only one chemical compound. On the closer analysis, most of the components in essential oils have a limited variation of hydrocarbon skeletons attached with different degrees of unsaturation or additional of functional groups such as –OH (alcohols or phenols),- OR (ethers), carbonyl, ester and a few of nitrogen and sulphur. Thus, to classify the essential oils constituent is by the structure of carbon skeleton. The first classes of most represented compound found in essential oils is terpenoid compounds, as an example of the compounds shown in Table 2.2.

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