# EXTRACTION OF *ZINGIBER ZERUMBET (L) SMITH* OIL BY USING SOXHLET EXTARCTION METHOD

**WAHIDA BINTI AMAT FADZIL**

A report submitted in partial fulfillment of the
requirement for the award of degree the of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

DECEMBER 2010

# ABSTRACT

In this research, the soxhlet extraction method was used to extract oil from *Zingiber Zerumbet (L) Sm*ith rhizomes. The main chemical component in the oil which is zerumbone, has the potential as anticancer, anti-HIV and anti-inflammatory. The examined parameters were investigated are the effect of solvent and extraction time on extraction yield. The solvent used were dichloromethane, benzene and methanol. The extraction time was varied for 4 hr, 6 hr, 8 hr and 10 hr. Then the oil extracts obtained were evaporated using rotary evaporator to get concentrated oil. Then, the extracted oil was analyzed using GCMS to determine the oil composition. GCMS analysis result shows that extracted oil contain zerumbone and α-caryophyllene. Extraction using methanol produced higher yield compared to another solvent. The best extraction time is 6 hours extraction.

# ABSTRAK

Di dalam penyelidikan ini, kaedah pengekstrakan soxhlet telah digunakan untuk mengekstrak miyak daripada rizom *Zingiber Zerumbet (L) Smith.* Component kimia utama yang terkandung di dalam minyak adalah *zerumbone,* yang mempunyai potensi sebagai anti-kanser, anti-HIV dan anti-inflamasi. Parameter yang telah dikaji adalah kesan pelarut dan masa pengekstrakan terhadap hasil pengekstrakan. Pelarut yang digunakan adalah dichloromethane, benzene dan methanol. Masa pengekstrakan divariasikan kepada 4, 6, 8 dan 10 jam. Seterusnya, minyak yang diperolehi diruap menggunakan *rotary evaporator* untuk mendapatkan minyak yang pekat. Minyak yang diperolehi dianalisis menggunakan GCMS untuk mengenalpasti komponen yang terdapat di dalam minyak. Hasil analisis daripada GCMS menunjukkan minyak yang diekstrak mengandungi z*erumbone* and *α-caryophyllene*. Pengekstrakan menggunakan methanol menghasilkan hasil yang paling tinggi berbanding pelarut lain. Masa pengekstrakan yang paling baik adalah selama enam jam.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| GC | - | Gas Chromatograph |
| MS | - | Mass Spectrometer |

## LIST OF SYMBOLS

| | | |
|---|---|---|
| W1 | - | weight of the extract after evaporation |
| W2 | - | weight of dry solid |

## LIST OF APPENDICES

# CHAPTER I

# INTRODUCTION

## 1.1 Research Background

*Zingiberaceae* is one of the largest families of the plant kingdom. *Zingiberaceae* are distributed mostly in tropical and subtropical areas. The center of distribution is in South East Asia region. The greatest concentration of genera and species is in the Malesia region which is consist of Indonesia, Malaysia, Singapore, Brunei and Papua New Guinea (Sirirugsa, 1999). The *Zingiberaceae* is an economically important plant family in the tropics. Presently about 47 genera and nearly 2,000 species are recognised world wide, most of them in tropical areas, especially in Indo-Malaysia.

*Zingiber zerumbet (L) Smith* well known as *lempuyang*, is a wild ginger belonging to the *Zingiberaceae* family, and has been used as an ingredient in some traditional medicines. It is used in local traditional medicine as a cure for swelling, sores and loss of appetite. The juice of the boiled rhizomes has also been used as a medicine for worm infestation in children.

From the previous study, the important chemical content in the *Z. zerumbet's* oil is *zerumbone*. *Zerumbone* is a sesquiterpene phytochemical with potential anticancer, anti-intiflammatory, anti-HIV and other biological activities, most abundantly found in *Z. zerumbet*. The volatile oils of the rhizomes have been shown to contain *zerumbone, humulene* and *camphene* (Nik-norulaini *et al.*, 2009).

There are several methods to extract essential oil from herb and spices like steam distillation, hydrodistillation, and solvent extraction. In this study, the focus is one of the solvent extraction methods which are soxhlet extraction method. The soxhlet extraction method presented a good yield of components in extract. The works flow involves preparation of the material include sample collection, drying, grinding, extraction process and analyze the oil composition.

## 1.2 Problem Statement

Herbal medicine becomes more popular among the people who looking for natural and chemicals or drugs free medicine. Plant essences and extracts that have developed into modern essential oils have been used for centuries. The variety of uses for essential oils and their components is very broad and is determined by their chemical physical and sensory properties.

Malaysia is the world's 12 mega diversity country that rich in biological resources especially plants with medicinal properties. There are 15,000 known vascular plants and 1200 of the number is medicinal plant and the rest is aromatic plant. Establishment of plantations of medicinal and aromatic plants in Malaysia such as *Eurycoma longifolia* (tongkat ali), *Orthosiphon stamineus* (misai kuching), Centella asiatica (pegaga), Cymbopogon nardus (serai wangi) and *Jasminium sambac* (jasmine).

*Z. zerumbet* has been used for a long time, in terms of its uses, it is well established. Its rhizomes are used in local traditional medicine as a cure for swelling, sores and loss appetite. The juice of the boiled rhizomes has also been used as medicine for worm infestion in children (Nik-Norulaini *et al.*, 2009; Faizah *et al.*, 2002). *Z. zerumbet* is most widely known around the worlds as the shampoo ginger (Baby, 2009).

The main component in the rhizomes, *zerumbone* has the potential to be used in the treatment of Alzheimer's disease (Bustaman *et al.*, 2008). *Z.zerumbet* also showed potential to suppress tumor activity (Murakami *et al.*, 2002), anti-inflammatory (Chien *et al.*, 2008; Mukarami *et al.*, 2004). Recent progress in understanding the anticancer properties of *zerumbone* and the increasing public interest in health will lead to the demand for large amounts of *zerumbone* in the future (Yu *et al.*, 2008).

Besides having medicinal properties, the extract from the *Z. zerumbet* can contribute to Malaysian economy. With *Z. zerumbet's* endless versatility and impressive medicinal properties, has received considerable attention because of the pharmacological significance of zerumbone. This is why *Z. zerumebet* become our interest in this research in order to accomplish the objective mentioned earlier.

In this research, the method used is soxhlet extraction method. Even though there are some research said that this method consuming time and solvent used, but according to other research, the soxhlet extraction method presented a good yield of components in extract (Ahmad *et al.*, 2010).

## 1.3 Objective

The purposes of this study are to determine the best condition to *Z. zerumbet* oil in terms of solvent use and extraction time for extraction process and to determine the compounds available in the extracted *Z. zerumbet* oil.

## 1.4 Scope of Research

This research is an experimental study of soxhlet extraction using rhizomes of *Z. zerumbet* as raw material.

i. Investigate the effect of solvent nature on extraction. The solvent used in this study are: benzene, dichloromethane and methanol.

ii. Investigate the effect of extraction time on extraction. The experiment was operated at 4, 6, 8 and 10 hours.

iii. Analyze the oil composition from extraction process by using Gas Chromatography-Mass Spectrometer.

## 1.5 Rationale and Significant of Study

Based on the knowledge of operating parameters for the extraction of *Z. zerumbet* oil gained through this research, it will enable to increase the extraction yield by monitoring parameters so that the pure oil can be used effectively.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

### 2.1.1 The *Zingiberaceace* Family

Species of the *Zingiberaceae* are the ground plants of the tropical forests. They mostly grow in damp and humid shady places. they also found infrequently in secondary forest. Some species can fully expose to the sun, and grow on high elevation. *Zingiberaceae* is one of the largest families of the plant kingdom. It is important natural sources that provide many useful products for food, species, medicines, dyes, perfume and aesthetics to man. *Zingiberaceae* are distributed mostly in tropical and subtropical areas. The center of distribution is in South East Asia region. The greatest concentration of genera and species is in the Malesian region which is consist of Indonesia, Malaysia, Singapore, Brunei and Papua New Guinia (Sirirugsa, 1999).

The rhizomes of *zingiberaceae* family are a vegetable widely used in many asian countries and their medicinal function have been broadly discussed (*Chen et al.*, 2008). The common *Zingiberaceae* species are easily recognised because they are used as food flavour, mixtures in traditional medicine as well as ornamental plants. *Zingiber zerumbet (L) Smith* well known as *lempuyang*, is a wild ginger belonging to the Zingiberaceae family

### 2.1.2 Zingiber Zerumbet (L) Smith

*Zingiber Zerumbet (L) Smith* is a wild ginger, grows in wide ranges around Southeast Asia. The rhizomes of the plant are employed as a traditional medicine for anti-inflammation and the like in some restrict (Jang, 2004; Farnsworth and Bunyapraphatsara, 1992).

*Z. zerumbet* grows to about 7 ft (2.1 m) tall with long narrow leaves arranged oppositely along the stem. The inflorescence is borne on a separate pseudostem from the leaves. It is a spike; bracts subtend the position of each of the flowers giving the inflorescence its pinecone shape. The Figure 2.1 shows the plant of *Z. zerumbet.*

The part utilize of *Z. zerumbet* is its rhizomes. According to Akiyama (2006) rhizomes of many plants belonging to the genus *Zingiber (Zingiberaceae)* are used as spices or traditional folk medicines in many parts of the world. Its rhizomes are pale yellow color with pungent smell. Figure 2.2 shows the rhizomes of *Z. zerumbet*.

**Figure 2.1:** *Zingiber Zerumbet( L) Smith* Whole Plant (http:// herbalmiracles. blogspot.com)

**Figure 2.2:** *Zingiber Zerumbet (L) Smith* Fresh Rhizomes (http:/ / addthailand.com)

### 2.1.3 Application of *Zingiber Zerumbet (L) Smith*

*Z. zerumbet* has been used as a traditional medicine for many years. *Z. zerumbet* is used in local traditional medicine as a cure for swelling, sores and loss appetite. The juice of the boiled rhizomes has also been used as medicine for worm infestion in children (Nik-Norulaini *et al.*, 2009; Faizah *et al.*, 2002). *Z. zerumbet* is most widely known around the worlds as the shampoo ginger (Baby, 2009). It is in fact used as a shampoo in Asia and Hawaii, and is one of the ingredients in several commercial shampoos. For toothache, the cooked and softened rhizome is pressed into the cavity to reduce the pain (tropilab.com). In Indonesia, it is traditionally used as the main ingredient in *jamu* manufacturing (Riyanto, 2007). In Thailand, they used the rhizomes to relieve stomach ache, macerated in alcohol is regarded as tonic and also as the spice ginger. The flowers are eaten as vegetable (Sirirugsa, 2009).

There are many research have been done in order to commercialize the value extracted from *Z. zerumbet*. These researches focused on the ability of *zerumbone* to treat several diseases. The *zerumbone* has the potential to be used in the treatment of Alzheimer's disease (Bustaman *et al.*, 2008). *Z. zerumbet* also showed potential to suppress tumor activity (Murakami *et al.*, 2002). In addition, the extract shows other pharmacological activities such as anti-inflammatory (Chien *et al.*, 2008; Mukarami *et al.*, 2004).The derived from the bioactive compound of *Z. zerumbet* also has shown lesser anti tumor effect towards cancer cell (Wahab *et al.*, 2008).

## 2.2 *Zingiber Zerumbet (L) Smith* Essential Oils

### 2.2.1 Constituent in *Z. Zerumbet* Essential Oil

An essential oil is a liquid that is generally distilled from the leaves, stems, flowers, bark, roots, or other elements of a plant. Essential oils, contrary to the use of the word 'oil' are not really oily-feeling at all. Most essential oils are clear, but some oils such as patchouli, orange and lemongrass are amber or yellow in color.

The *Z. zerumbet* oil contains several of volatile compounds like *zerumbone, α-Caryophylleno,* and *camphene*. There are several previous researches reported about the high content of *zerumbone* in the oils. It is reported that volatile oils of *Z. zerumbet* from Malaysia and southern India consist of high content of *zerumbone* in the oil (Baby *et al.,* 2009). The Malaysian accession recorded the content of the *zerumbone* in the oil is about 68.9% while the southern Indian accession of *Z. zerumbet* is 76.3% - 84.8% zerumbone content in their rhizomes oils. The detail content of the rhizomes oil can is viewed in Appendix A.

The volatile compounds in the rhizomes oils from Bangladesh also were identified by GCMS in previous study and tabulated in Appendix B. The research studied about the content of oil in the leaf and rhizome of *Z. zerumebet*. It is reported that twenty-nine component have been determined in the leaf oil and thirty component in the rhizomes oil (Bhuiyan, 2009).

Other than oil from the rhizomes, the extraction of the oil from the leaves and flowers also can be commercialized. It is because, the oil from these two parts reported contain *(E)-nerolidol, beta-caryophyllene* and linalool. (Chane-Ming *et al.,* 2003) *E-nerolidol* and *Linalool* has many application based on its pleasant scent in the manufacturing fragrance or flavors.

### 2.2.2 *Zerumbone*

*Zerumbone* is a sesquiterpene phytochemical from *Z.zerumbet* oil. The chemical formula of *zerumbone* is $C_{15}H_{22}O$ and the molecular weight of this structure is 218.340 g/mol. The IUPAC name of *zerumbone* is (2E,6E,10E)-2,6,9,9-tetramethylcycloundeca-2,6,10-trien-1-one. The other synonyms name are (E,E,E)-2,6,9,9-tetramethyl cycloundeca-2,6,10-trien-1-one and (E,E,E)-2,6,9,9-tetramethyl-2,6,10-cycloundecatrien-1-one.

**Table 2.1:** Properties of *Zerumbone* (http://www.thegoodscentscompany.com)

| | |
|---|---|
| Boiling Point | 321-33°C at 760mmHg |
| Flash Point | 133.33 °C |
| Density | 0.887± 0.06 g/cm$^3$ |
| Polarizability | 27.11± 0.5 x10$^{-4}$ cm$^3$ |

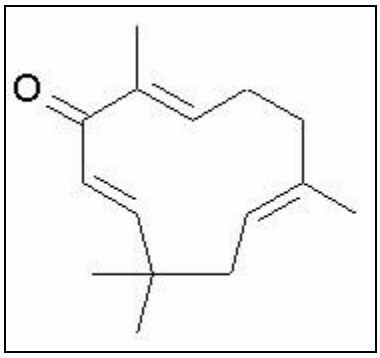


**Figure 2.3:** Structure of *Zerumbone* **(**Wahab *et al.*, 2008)

*Zerumbone* contains three double bonds; an isolated one at C2, and two at C6 and C9 which are part of a cross conjugated dienone system. Of these the C6 double bond appears least hindered, being furthest from the gem-dimethyl substituents at C11. (Kitayama *et al.*, 1999)

## 2.3 Soxhlet Extraction

### 2.3.1 Solvent Extraction

Solvent extraction is commonly known as solid-liquid extraction. Solid-liquid extraction (leaching) is the process of removing a solute or solutes from a solid by using of liquid solvent. Soxhlet is one of the leaching techniques mostly used for a long time (Luque de Castro & Garcia-Ayuso, 1998).

### 2.3.2 Introduction

The Soxhlet extractor is named after Franz Ritter von Soxhlet, German chemist of Belgian. Soxhlet extraction is used widely in the extraction of plant metabolites because of its convenience. This method is adequate for both initial and bulk extraction. The soxhlet extractor is used to extract organic compounds from solid material. The concept of the soxhlet extraction is organic compound are extracted by repeated washing with an organic solvent under reflux in a special glassware. In general, the setup consists of round bottom flask containing the solvent, an extraction chamber and a condenser (Brill *et al.,* 2006). Typically, the solid are the consistency of small particle like powder or soil. It is stated in several extraction studies, the raw materials are grounded before the extraction can be preceded (Tewtrakul *et al.*, 1997; Alfaro *et al.*, 2002). The smaller size of the material will increase the mass transfer of active component into the solvent.

The general operation of the soxhlet extraction is the solvent generated by heating the boiling flask will condense and is allowed to drip back onto the thimble. The liquid condense that drips out onto the sample perform the extraction which then passes through the container and back into boiling flask. The cycle is repeated continuously as long as needed. As it progress, the oil is concentrated in the flask

### 2.3.3 Advantages of Soxhlet Extraction

According to previous study on soxhlet extraction method, there are most outstanding advantages of this conventional extraction method. In the soxhlet extraction, sample is repeatedly brought into contact with fresh solvent, thereby helping to displace the transfer equilibrium. The temperature of the system remains high since the heat applied to distillation flask reaches the extraction capacity to some extent.

Furthermore, no filtration is required after the extraction process. Soxhlet extraction method also is a very simple methodology which needs little specialized training since the basic equipment is inexpensive. This conventional method also has the possibility to extract more sample mass compare to other methods like micro-wave extraction. (Luque-Garcia & Luque de Castro, 2004) Based on the advantages of the soxhlet extraction, this conventional method has been a standard leaching technique in extraction process.

### 2.3.4 Disadvantages of Soxhlet Extraction

The disadvantages of this method are a large solvent consumption as well as a long sample treatment (Alfaro *et al.*, 2003; Luque de Castro & Garcia-Ayuso, 1998). The large amount used can cause large amount of solvent wasted which not only expensive to dispose and also can cause additional environmental problems.

## 2.4 Effect of Solvent in Extraction

Solvent can be classified as polar and non-polar solvent. Polar solvents have molecules whose electric charges are unequally distributed, leaving one end of each molecule more positive than the other. Non polar solvents have molecules whose electric charges are equally distributed and are not miscible with water. Polar reactants will dissolve in polar solvents. Non-polar solvents dissolve non-polar compounds best. Table 2.4 shows the Properties of some common solvents used in products extraction.

**Table 2.2:** Physicochemical Properties of Some Common Solvents Used in Products Extraction (Sarker *et al.*, 2006)

| Solvent | Polarity | Polarity Index | Boiling Point (°C) |
|---|---|---|---|
| n-Hexane | Non-polar | 0.0 | 69 |
| Dichloromethane | Polar | 3.4 | 41 |
| Benzene | Non-polar | 3.0 | |
| n-Butanol | Polar | 3.9 | 118 |
| iso-propanol | Polar | 4.0 | 82 |
| Chloroform | Non-polar | 4.1 | 61 |
| Ethyl Acetate | Polar | 4.4 | 77 |
| Acetone | Polar | 5.1 | 56 |
| Methanol | Polar | 5.1 | 65 |
| Ethanol | Polar | 5.2 | 78 |
| Water | Polar | 9.0 | 100 |

In this study four different solvents are used: benzene, dichloromethane and methanol. Among the solvent used, benzene is the non polar solvent and the others are polar solvent. It is stated in a literature that *zerumbone* is a polar solvent therefore a polar solvent would be the best for extraction (Hasham *et al.*, 2003).

In a research by Kumoro *et al.*, (2009) have shown the effect of polarity of solvents in soxhlet extraction. The result of the study indicated that high extracted yield was obtained from extraction employing polar organic solvent containing hydroxyl group (methanol or ethanol). Low extract yield when extraction was done using non polar solvent.

A research on one of the *zingiberaceae* species using different solvent had been conducted by Riyanto (2007). In his work, extraction of *Zingiber amaricans BL*. rhizomes was done by using four different solvents which are hexane, dichloromethane, methanol and acetone. The work was done by using two different methods: maceration and soxhlet extraction. In this research, author did not state which solvent give the best performance. It only showed that by using these four solvents, the extracted contains the isolated compound from rhizomes which is *zerumbone*.