EXTRACTION AND CHARACTERIZATION OF ESSENTIAL OIL FROM GINGER (*ZINGIBER OFFICINALE* ROSCOE) AND LEMONGRASS *(CYMBOPOGON CITRATUS)* BY MICROWAVE-ASSISTED HYDRODISTILLATION(MAHD)

RANITHA A/P MATHIALAGAN

UNIVERSITI MALAYSIA PAHANG

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JUDUL : **EXTRACTION AND CHARACTERIZATION OF ESSENTIAL OIL FROM GINGER (*ZINGIBER OFFICINALE* ROSCOE) AND LEMONGRASS (*ZYMBOPOGON CITRATUS*) BY MICROWAVE ASSISTED HYDRODISTILLATION (MAHD)**

SESI PENGAJIAN : 2011/2012

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Associate Prof. Dr. Abdurahman Hamid Nour
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RANITHA A/P MATHIALAGAN

Thesis submitted in fulfillment
of the requirements for the award of degree of
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JANUARY 2012

SUPERVISOR'S DECLARATION

"I hereby declare that I have read this thesis and in my opinion this thesis has fulfilled the qualities and requirements for the award of Degree of Bachelor of Chemical Engineering"

Signature : ……………………………………………………………..

Name of Supervisor : ASSOCIATE PROF. DR ABDURAHMAN HAMID NOUR

Date : 3 DECEMBER 2010

STUDENT'S DECLARATION

I declare that this thesis entitled "Extraction and Characterization of Essential Oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* by Microwave-assisted Hydrodistillation(MAHD)" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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Name : RANITHA MATHIALAGAN

Date : 16 JANUARY 2012

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ABSTRACT

Microwave-assisted hydrodistillation (MAHD), an advanced distillation method that take advantage of microwave heating with the conventional hydrodistillation, recently been widely attended for the extraction of essential oil from medicinal plants and herbs due to its economic and green technology. One way in maximizing the efficiency of a method is optimizing its parameter condition to obtain maximum yield. In that case, this study was carried out to investigate the performance of MAHD in the extraction of essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass (*Cymbopogon citratus).* The effect of three main factors which were microwave power, extraction time and water to raw material ratio were investigated to optimize the extraction operating conditions for obtaining maximum oil yield. As a result the best condition that has been determined for maximum essential oil production were under 250 W microwave power for 90 minutes at water to raw material ratio of 8:1. This optimum condition was finalized based on its maximum yield from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass (*Cymbopogon citratus)* which were 0.85% (w/w) and 1.37%(w/w), respectively. Then, the oil samples at different extraction time were analyzed to evaluate its quality by determined its chemical constituent through GC-MS. The main components detected in the essential oil of Ginger (*Zingiber Officinale* Roscoe) were Borneol, β-Bisabolene, Cineole, α-Cedrene, α-Curcumene, β-Farnesene (E), β-Sesquihelladiene, β-Thujene and Zingiberene. Wherelse, the main components in the essential oil of Lemongrass (*Cymbopogon citratus)* were Citral, Geranic Acid, Geranyl Acetate, Linalool, Neric acid, (Z) Citral, β-mycrene and β-Thujene. The dominant component in the essential oil of Ginger (*Zingiber Officinale* Roscoe) and Lemongrass (*Cymbopogon citratus)* were Zingiberene and Citral, respectively. The maximum oil yield from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* were obtained at shorter extraction period without significant affect on their chemical constituents. This may prove MAHD as a cost effective method and also expected would bring volume of worth in the essential oil production industry.

ABSTRAK

Pergabungan microwave dengan penyulingan berasaskan air (MAHD) adalah suatu teknlogi terkini yang menggunakan kelebihan gelombang mikro dalam aplikasi penyulingan hidro tradisional yang sejak kebelakangan ini digunakan secara berleluasa dalam pengekstrekan minyak pati tumbuhan herba kerana kosnya yang rendah serta teknologinya yang mesra alam. Salah satu cara untuk memaksimakan kebolehan sesuatu kaedah adalah dengan mengoptimumkan faktor-faktor yang mempengaruhi penghasilan minyak pati untuk memperoleh hasil yang tinggi.Oleh yang demikian, penyelidikan ini telah dijalankan untuk menyiasat kebolehan MAHD dalam pengekstrekan minyak pati halia dan serai. Tiga fakto utama iaitu kuasa gelombang mikro, masa ekstrasi dan nisbah air kepada bahan mentah telah dikaji untuk mengenalpasti kombinasi terbaik untuk menghasilkan jumlah minyak pati yang tinggi.Dengan itu, kombinasi pembolehubah yang memberikan hasl minyak yang terbanyak telah dikenalpasti sebagai kuasa gelombang mikro 250W, masa pengekstrekan selama 90 minit dan nisbah air kepada bahan mentah 8:1. Keadaan ini boleh digunapakai untu kedua-dua tumbuhan yang dianalisa dalam penyelidikan ini. Kombinasi kedaan pembolehubah ini telah dimuktamadkan sebagai keadaan yang teroptimum berdasarkan jumlah minyak yang dihasilkannya iaitu 0.85%(w/w) bagi halia dan 1.37% bagi serai. Kemudian, minyak pati yang dihasilkan telah dianalisa dengan menggnakan GC-MS untuk mengenalpasti komponen-komponen kimia yang terkandung dalam miyak tersebut bagi mengetahui paras kualitinya. Komponen-komponen kimia utama yang dikenalpasti dalam minyak pati halia adalah Borneol, β-Bisabolene, Cineole, α-Cedrene, α-Curcumene, β-Farnesene (E), β-Sesquihelladiene, β-Thujene dan Zingiberene. Manakala komposisi utama minyak pati serai adalah Citral, (Z) Citral, β-mycrene dan β-Thujene. Komponen yang dominan bagi halia adalah zingiberene dan bagi serai adalah citral. Hasil minyak pati yang tinggi telah tercapai dalam masa pengekstrekan yang rendah melalui kaedah MAHD ini tanpa sebarang penjejasan keatas komposisi kimianya. Ini membuktikan MAHD sebagai suatu kaedah pengekstrekan yang berkos rendah serta ia diramalkan dapat membawa banyak kebaikan dalam indutri penghasilan minyak pati.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

˚ C	Degree Celsius
%	Percentage
kPa	Kilo-Pascal
Hz	Hertz
GHz	Giga-Hertz
W	Watts
ml	Mili-Liter
g	Grams
L	Liter
min	Minutes
hr	Hours
m	Meter
mm	Mili-Meter
µL	Micro-Liter
cm	Centimeter
mL/min	Mili-Liter Per Minute
w/w	Weight of Oil/Weight of Plant Materials
$	Dolar
RM	Ringgit Malaysia
V	Voltage
α	Alpha
β	Beta

LIST OF ABBREVIATIONS

HD	Hydrodistillation
MAHD	Microwave-Assisted Hydrodistillation
GC-MS	Gas Chromatography-Mass Spectrometer
MAE	Microwave-Assisted Extraction
FC	Foot Cell
SC	Stalk Cell
MHC	Mother Headcell
HC	Head Cell
GC	Gas Chromotograph
MS	Mass Spectrometer
EID	Electron Impact Deionization

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Essential oil referred to any concentrated, hydrophobic (immiscible with water), typically lipophilic (oil or fat soluble) liquid of plants that contains highly volatile aroma compounds and carries a distinctive scent, flavor, or essence of the plant. This large and diverse class of oils also is referred to as volatile oils or ethereal oils. Essential oils are found in diverse parts of plant including leaves, seeds, flowers, roots and barks. For the plant, essential oils are thought to be vital for the life of the plant, containing compounds that help to fight parasites and infections; many essential oils have anti-bacterial, anti-fungal, and anti-parasitic properties. For people, essential oils are used in perfumes, cosmetics, and bath products, for flavoring food and drink, for scenting incense and household cleaning products, and for medicinal purposes. Interest in essential oils has revived in recent decades, with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects.

Zingiber Officinale Roscoe or its common name ginger is a perennial herb and grows to about 3 - 4 feet high with a thick spreading tuberous rhizome. Every year it shoots up a stalk with narrow spear-shaped leaves, as well as white or yellow flowers growing directly from the root. The name ginger is said to be derived from Sanskrit word srngaveram meaning "horn root" with reference to its appearance. The plant is said to originate from India, China and Java, yet is also native to Africa and the West Indies. They are commonly used as ornamentals, as spices, and for their medicinal properties. Gingers are distinguished by the presence of a labellum, formed by the fusion of two sterile stamens, and by the presence of essential oils in their tissues. The characteristic odor and flavor of ginger is caused by a mixture of zingerone, shogaols and gingerols, volatile oils that compose one to three percent of the weight of fresh ginger. Ginger oil can vary in color from pale yellow to a darker amber color and the viscosity also ranges from medium to watery. The essential oil has various chemical constituents including the following which are a-pinene, camphene, b-pinene, 1,8-cineole, linalool, borneol, y-terpineol, nerol, neral, geraniol, geranial, geranyl acetate, b-bisabolene and zingiberene. The oil is extracted by means of steam distillation from the unpeeled or dried, ground-up root (rhizome) of the plant, and can yield about 2 - 4 % oil.

Cymbopogon citratus which is also commonly known as lemongrass, a native of India, is comes from family of fragrant grasses. *Cymbopogon* is a tall, aromatic perennial grass that can grow up to 90 cm in height and 5 mm wide. Fresh lemongrass contains approximately 0.4% volatile oil and rests are non-volatile components and nutritious such as calcium, iron, magnesium, manganese, phosphorus, potassium, selenium and zinc. Lemongrass essential oil is extracted from the fresh or partly dried leaves by steam distillation. It has a lemony, sweet smell and is dark yellow to amber and reddish in color, with a watery viscosity. The main chemical components of lemongrass oil are myrcene, citronellal, geranyl acetate, nerol, geraniol, neral and traces of limonene and citral. The essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* used for same purposes which for culinary and medicinal. Lemongrass oil also use in perfumes and as insect repellent.

The main methods to obtain essential oils from the plant materials are hydrodistillation (HD), steam distillation, steam and water distillation, maceration, empyreumatic distillation, and expression. Among these methods, HD has been the most common approach to extract the essential oils from the medicinal herbs plants. However, in order to reduce the extraction time and possibly improve the extraction yield, to enhance the quality of the approaches such as microwave-assisted extraction (MAE), pressurized solvent extraction, supercritical fluid extraction, and ultrasound-assisted extraction have also been sought. In an attempt to take advantage of microwave heating with the conventional HD, microwave-assisted hydrodistillation (MAHD) was then developed and used for the extraction of essential oils from some plants. Part of them are extraction of essential oils from *Satureja hortensis* and *Satureja Montana* (Rezvanpanah *et al*.,2008), Mango (*Mangifera indica* L.) flowers (Wang *et al.*,2010) and from *Thymus vulgaris* L. (Golmakani *et al.*, 2007). However, there is no research yet been carried out of extracting essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus) by* MAHD. Therefore, this study will be carry out to investigate the potential of MAHD for the extraction of essential oils from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* besides evaluating the extraction time and its cost.

1.2 PROBLEM STATEMENT

The worldwide market for essential oils has been estimated at US$2.6 billion, with an annual growth rate of 7.5 percent. It is projected that the value of the global market for herbal products would reach US$200 billion by the year 2008 (The Sun, 2001). In Malaysia, the herbal market was estimated to be worth RM2.5 billion annually, with the local herbal industry capturing only 5 percent to 10 percent of the market. Moreover, the herbal industry is expected to be the main contributor to the country's income in the future (Berita Harian, 2001).

This increasing demand of essential oil, such as ginger oil and lemongrass oil has opened up wide opportunities for global marketing and this leads to the requirement of competitive product in market which comes with all the advantages in term of cost, quality and its production time. As stated before essential oil is a volatile component. Therefore, it is vital to identify a best extraction technique, so that a higher quality essential oil with higher yield can be extracted.

Hydrodistillation (HD) is the most common approach to the extraction of essential oils from medicinal herbs and plants. However, these conventional methods present several drawbacks such as long extraction times, potential loss of volatile constituents, high energy use, and so on. Thus, it is not suitable with the current market requirement. Therefore, developing an alternative extraction technique that is rapid, sensitive, safe, and energy-efficient is highly desirable. As the result, to improve this existing extraction process, a more active and efficient enhancement can be added and microwave is one of them.

Microwave-assisted hydrodistillation (MAHD) method is a more recent technique used to recover volatile components such as essential oil. In this method, plant material placed in a Clevenger apparatus is heated inside a microwave oven for a short period of time to extract the essential oil where heat is produced by microwave energy. The sample reaches its boiling point very rapidly, leading to a very short extraction or distillation time. With the microwave distillation technique it is possible to achieve distillation with the indigenous water of the fresh plant material (Kürkçüoğlu, 2010).

Although the effect of microwave-assisted hydrodistillation has been conduct on various essential oil extraction, its effect on Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* , has not yet been explore yet and therefore this research will conducted. Further priority is given on the factors that can influence the extraction time and operation cost.

1.3 OBJECTIVES

The main objective of the present research are to identify the chemical composition of the essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* which were extracted by using microwae-assisted hydrodistillation method (MAHD). A second objective was to investigate the performance of MAHD on the yield of essential oil. Finally, the objective of this research is to identify the effect of extraction time and operation cost.

1.4 SCOPE OF STUDY

1.4.1 To analyze the chemical composition of essential oil by using Gas Chromotography- Mass Spectrometry (GC-MS).

1.4.2 To study the performance of microwave-assisted hydrodistilation method in extraction of essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus).*

1.4.3 To study the effect of extraction time to the yield of essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus).*

1.4.4 To find the extraction yield and efficiency of MAHD method.

1.4.5 To study the operational cost based on extraction time and yield.

1.5 RATIONALE AND SIGNIFICANCE

The rationale of this proposed research project is to examine the performance of Microwave-Assisted Hydrodistillation method in the extraction of essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* based on its yield and efficiency. The results of this research would signify the identification of a best extraction method for the production of essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* which demand is increasing from year to year in global market for numerous applications.

There are various advantages of using Microwave-Assisted Hydrodistillation method for this extraction purpose. Although the distillation was accomplished in a shorter time, an oil yield through this process is slightly high compared to the conventional extraction method and this would go well to supply the ever increasing rate of demand for essential oil from ginger and lemongrass. This shorter period consumption for extraction leads to lower power consumption and this reduce the operating cost as well. In addition, MAHD also doesn't utilize any chemicals. Therefore, the essential oil extracted from this method is essentially pure and safe. These criteria are very important for essential oil such as ginger oil and lemongrass oil since they are highly employed for culinary and medicinal purposes.

CHAPTER 2

LITERATURE REVIEW

2.1 ESSENTIAL OIL

Essential oils were mankind's first medicine. They have a long history, being used by the ancient civilizations of Egypt, Greece, India, and Rome; more than 5,000 years ago, the ancient civilizations of Mesopotamia utilized machines for obtaining essential oils from plants. Today modern science is rediscovering the wisdom of the ancients. Essential oils are able to reach deep into the recesses of our brains, cross over the chemical barriers, and open the hidden channels within our minds and bodies. Essential oils fragrances pass on to the limbic system of the brain without being registered by the cerebral cortex. Within the limbic system resides the regulatory mechanism of the innermost core of our being. Since the limbic system is directly connected to those parts of the brain that control heart rate, blood pressure, breathing, memory, stress levels, and hormone balance, essential oils can have some very profound physiological and psychological effects. An essential oil is a liquid that is generally steam or hydro-distilled from flowers, leaves, bark and roots of plants and trees and are the compounds responsible for the aroma and flavor associated with herbs, spices, and perfumes.

Essential oils may have two major components which are terpene hydrocarbon, and oxygenated compounds terpene hydrocarbon can be divided into two group; monoterpenes and sesquiterpenes. While oxygenated compounds are phenols, monoterpenes, and sesquiterpenes alcohols, aldehydes, ketons, esters, lactones, coumarins, ethers, and oxides. Monoterpenes compounds are found in nearly all essential oil and have a structure of 10 carbons atoms and at least one double bond. The 10 carbons atoms are derived from two atoms isoprene units. Monoterpenes react readily to air and heat sources. These components have anti-inflammatory, antiseptic, antiviral, and antibacterial therapeutic properties. Sesquiterpenes consist of 15 carbons atoms and have complex pharmacological actions. It has anti-inflammatory and anti-allergy properties. There are three main aromatic groups which are phenols, terpenes alcohols, and aromatic aldehydes.

The essential oils in aromatic herbs are known to be largely located within the glandular structures that develop on the surface of leaves and other organs of the plants. The peltate hairs appear to contain most of the oil and are henceforth called 'the glands'. Each gland originates from a single protodermal cell that undergoes division and derives two unequally sized cells. The lower cell corresponds to the foot cell (FC) while the upper daughter cells re-divides to yield the stalk cell (SC) and the mother headcell (MHC). The foot and stalk cell remain unicellular throughout the subsequent development of the gland while the mother cell of the head further divides to give rise to 8 or 12 head cells (HC) by the end of the development. Climatic factors, rates of plant metabolism, differentiation and secretory activity of glandular hairs affect synthesis and secretion of essential oils. Following figure illustrate the developments of these three glands.

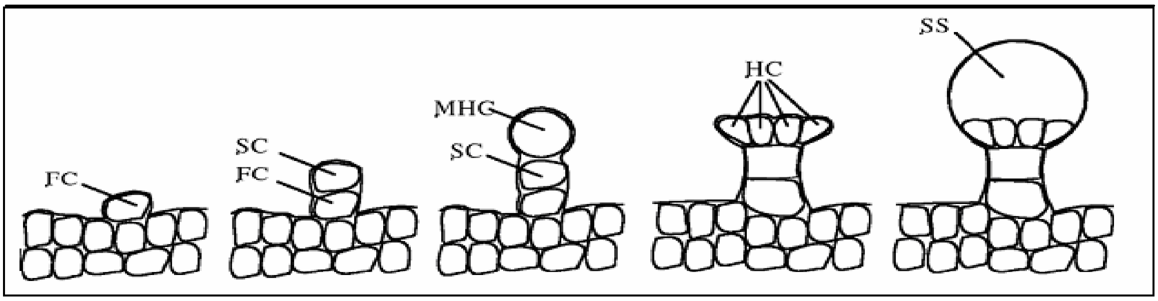


Figure 2.1: Developments of glands

Source: James 2005

2.2 AN INTRODUCTION OF GINGER (*Zingiber Officinale* Roscoe)

The botanical name of ginger plants, as mentioned earlier, is *Zingiber officinale*. It is thought to come from the Sanskrit word *singabera* which was from Arabic and Greek words meaning 'shaped like a horn'. It probably got its name because the rhizomes look like deer's antlers. It is also known by various names such as African ginger, black ginger, sunthi, East Indian pepper, Jamica pepper, German ingwer, Italian zenzero jengibre, myoga, zangvil, gingembre, dinnsear, engifer, shouga, imbir, luya and gung.

Table 2.1: Taxonomy of Ginger (*Zingiber Officinale* Roscoe)

Kingdom	*Plantae*
Subkingdom	*Viridaeplantae*
Phylum	*Tracheophyta a*
Subphylum	*Spermatophytina*
Intraphylum	*Angiosperma*
Division	*Magnoliphyt*
Class	*Liliopsida*
Order	*Zingiberales*
Family	*Zingiberaceceae*
Genus	*Zingiber*
Species	*Officinale*
Scientific name	*Zingiber officinale*
Common name	Ginger

Source: Farlex (2004)

An essential oil is a liquid that is generally steam or hydro-distilled from flowers, leaves, bark and roots of plants and trees and are the compounds responsible for the aroma and flavor associated with herbs, spices, and perfumes. Essential oils molecules are made up primarily of carbon, hydrogen, and oxygen. The aromatic constituents of essential oils are built from hydrocarbon chains. The basic building block of many essential oils is a five-carbon molecule called an isoprene which built most of the essential oils.

The part of the Ginger (*Zingiber Officinale* Roscoe) plant commonly known and consumed is the underground stem, or rhizome, although it is often referred to as "ginger root". This part of the plant stores its food reserves, and is the one used for both cooking and medicinal purposes. The stem grows up to about 12 inches above the ground and has long, ribbed, green leaves, with yellow or white flowers. Ginger flowers have also been described as being greenish yellow and streaked with purple down the sides.

The strong taste and stimulating effects of Ginger (*Zingiber Officinale* Roscoe) on the body are largely down to the presence of an oily substance called gingerol as well as volatile oils. Gingerols and shogaols present in Ginger (*Zingiber Officinale* Roscoe) as pungent chemical substances. Ginger (*Zingiber Officinale* Roscoe) also contains some amount of essential oils in the root, which is the reason for its fragrance.

To grow Ginger (*Zingiber Officinale* Roscoe), the rhizome is simply planted in the ground and a new plant springs up. Ginger can actually grow in many places, but moist regions near the equator are considered best. As ginger ages, the amount of essential oils increases. So, the intended use of the rhizome determines when it is harvested. If it is for use as fresh or preserved ginger, it might be harvested when it is about 5 months old where at this time the plants have not yet matured. The rhizomes are still tender and not quite as pungent. Dried ginger calls for a more pungent aroma so those plants might be harvested at 8 or 9 months. If it is the essential oils that one is after, the plant might be harvested even after 9 months. Ginger is traditionally harvested by hand although there are mechanical diggers made just for this purpose. China is said to be largest producer of ginger today, followed by India.

Figure 2.2: Schematic Diagram of Ginger (*Zingiber Officinale* Roscoe)

Source: Paul Gates 2008

2.2.1 History of Ginger (*Zingiber Officinale* Roscoe)

The scientific name of Ginger which is *Zingiber officinale* was given by the English botanist, William Roscoe (1753-1831) in an 1807 publication. The history of Ginger goes back over 5000 years when the Indians and ancient Chinese applied it as a tonic root for all ailments. This proved when referred back in the Hindu epic Mahabharata written around the 4th century BC describes a meal where meat is stewed with ginger and other spices. It was also an important plant in the traditional Indian system of Ayurvedic medicine.

Besides that, since 2000 years ago ginger was also highly important as an article of trade and was exported from India to the Roman Empire where it was valued more for its medicinal properties than as an ingredient in cookery. It continued as an article of trade to Europe even after the fall of the Roman Empire, with Arab merchants controlling the trade in ginger and other spices for centuries.

Along with black pepper, ginger was one of the most commonly traded spices during the 13th and 14th centuries. Arabs carried the rhizomes on their voyages to East Africa to plant at coastal settlements and on Zanzibar. During this time in England, ginger was sought after, and one pound in weight of ginger was equivalent to the cost of a sheep.

2.2.2 Chemical Constituents of Ginger (*Zingiber Officinale* Roscoe)

The chemistry of ginger is well documented with the respect to the oleoresin and volatile oil. More than 400 chemicals have been identified in ginger rhizome. Geography, age of rhizome at harvest and extraction methods determines the relative proportions of chemicals. Chemical constituents of ginger classified to volatile oils which constitutes (1-3%) bisabolene, cineol, phellandrene, citral, borneol, citronellol, geranial, linalool, limonene, zingiberol, camphene and mainly of zingeberene, nonvolatile pungent compounds oleo-resin constitute (4- 7.5%) mainly gingerols and followed by shogoal, then Phenol (gingeol, zingerone), Proteolytic enzyme (zingibain), Vitamin B6, Vitamin C, Calcium, Magnesium, Phosphorus, Potassium, Linoleic acid. (Mahdi, Andayani and Ishak , 2010). Essential oil of ginger can be varying in color from pale yellow to a darker amber color.

2.2.3 Application of Ginger (*Zingiber Officinale* Roscoe)

Ginger (*Zingiber Officinale* Roscoe) is an interesting spice with many medicinal and culinary uses. A popular spice over 5000 years ago, it is still popular today and used in the cooking of both Eastern and Western cultures.

2.2.3.1 Health Benefits

Ginger (*Zingiber Officinale* Roscoe) is a remarkable remedy with a wide range of actions that are suitable for men, women and children. It is very safe and has a record of use that extends back thousands of years, across many cultures and continents. Followings are the list of some beneficial application of ginger for some health problems (Charypravi, 2010):

- **Inflammation:** Ginger is said to contain a substance called ‘zingibain’ which is analgesic in nature and can reduce pain caused by arthritis, muscle aches, rheumatoid arthritis.
- **Kidney:** Ginger root juice is able to dissolve kidney stones.

- **Impotency:** Ginger is helpful for men's health as well. Since ginger root and its oil are aphrodisiac in nature, it is effective in removing impotency and treating premature ejaculation.
- **Stress:** Ginger oil, being an essential oil is stimulating and therefore relives depression, mental stress, exhaustion, dizziness, restlessness and anxiety.
- **Malaria:** Ginger root and ginger oil is also effective against yellow fever and malaria.
- **Aromatherapy:** In aromatherapy, it is used to ease sore throat and also to clear up the moisture from runny noses. Ginger oil is also used to encourage sweat in case of fever.
- **Respiratory:**Most of us do not like the pungent smell of ginger, but this smell is useful for treating cold and cough.
- **Food Poisoning:** Ginger oil contains antiseptic as well as carminative properties that are useful in curing problems related to food poisoning.
- **Digestive System:** Its help to relief from stomach ache, indigestion, diarrhea, dysentery, intestinal infection, colic, spasms and also bowel related problems.
- **Heart Diseases:** Ginger essential oil is said to boost the cardiovascular system, thus helping in the prevention of a heart attack by decrease cholesterol

2.2.3.2 Toxicology

When used in the recommended doses, the side effects of ginger are rarely felt. This may not be true for all individuals, and some individuals may be very sensitive to the taste of the herb or they may experience symptoms such as heartburn when using the ginger. Ginger can be taken by individuals with a history of gallstones only after careful consultation with a nutritionally oriented doctor as side effects are possible. No safety issues seem to exist in the short-term use of ginger to treat the nausea and vomiting during a term of pregnancy. At the same time, the use of ginger as an herbal remedy in the long-term during the pregnancy is not advised as regular use can trigger side effects in the woman. The use of any ginger remedy by the patient before surgery must be made known to the doctor and the surgeon must be informed if the patient intends to undergo any form of surgery, this step must be taken to counteract the possible post anesthesia nausea in the patient (Herbs, 2000).

2.3 AN INTRODUCTION OF LEMONGRASS (*Cymbopogon citratus)*

Cymbopogon citratus, generally known as lemongrass is a fragrant tropical grass. It is thought the name lemongrass given due to its fresh stalks and leaves which have a clean lemon like odour. This is because it also contains an essential oil, which is also present in lemon peel. Lemongrass *(Cymbopogon citratus)* also known by different names in various languages such as (Agro Products, 2008):

- French : Citonelle
- Indian : Bhustrina, sera
- German : Zitronengras
- Malay: Serai
- Italian: Erba di limone
- Spanish: Hierba de limon
- Sinhalese : Sera
- Indonesian : Sere/Se

Lemongrass *(Cymbopogon citratus)* is a member of the *Poaceae (Gramineae)* plant family, of Indian origin, which includes palmarosa *(Cymbopogon martinii)* and citronella *(Cymbopogon nardus)*. There are 55 species of lemongrass, and the chemical components of each vary, although *citral* is the main component present in all varieties of lemongrass essential oil. In general, the West Indian Lemongrass or the East Indian Lemongrass species are used as essential oils in aromatherapy, although *Cymbopogon flexuous* has been used in aromatherapy too (Sharon Falsetto, 2009). The scientific classification of Lemongrass *(Cymbopogon citratus)* is further detailed in Table 2.2:

Table 2.2: Taxonomy of Lemongrass *(Cymbopogon citratus)*

Kingdom	*Plantae*
Intraphylum	*Angiosperms*
Division	*Monocots*
Class	*Commelinids*
Order	*Poales*
Family	*Poaceae*
Subfamily	*Panicoideae*
Tribe	*Andropogoneae*
Subtribe	*Andropogoninae*
Genus	*Cymbopogon*
Common name	*Lemongrass*

Source: Sharon Falsetto(2009)

Lemongrass *(Cymbopogon citratus)* is a tall fragrant perennial grass, throwing out dense fascicles of leaves from a stout rhizome. It can grow up to a height of 1 m. the leaves are sessile, simple, green, linear, equitantly arranged and can grow to an average size of 40 cm long x 1 cm wide. The plant bears leaves round the year and they are vivid bluish-green and when mashed they emit an aroma akin to lemons. The leaves of this plant are used for flavoring and also in the manufacture of medications. The leaves are refined by steam to obtain lemongrass oil - an old substitute in the perfume manufacturers' array of aroma. The oil of Lemongrass *(Cymbopogon citratus)* is yellow in color with a citrus/grass/lemon fragrance. This plant produces flowers at a much matured stage of growth. Thee rhizome is stout, creeping, robust and creamish yellow in section. The vertical rhizome produces branches, which then produces new suckers, which later will form new plantlets (Falina Saudi, 2008).

Lemongrass *(Cymbopogon citratus)* is propagated vegetatively through rooted slips. It can adapt itself in variety of soil and climate conditions. But, to get high oil content, Lemongrass *(Cymbopogon citratus)* should planted at well aerated soils with good fertility. This crop cannot withstand water logged and shady areas. Therefore it need open areas for its cultivation. The plants last three to four years and can be harvest after 60 days of planting. This crop is harvested about 20 cm above ground. There is no serious pest and disease problem faced by this crop. Only very damp conditions Lemongrass *(Cymbopogon citratus)* becomes more susceptible to insect pest. It is grown throughout Southeast Asia, Southern India, Sri Lanka, Central Africa, Brazil, Guatemala, the US and the West Indies, (Falina Saudi, 2008).

Figure 2.3: Lemongrass *(Cymbopogon citratus)*

Source: Sharon Falsetto, 2009

2.3.1 History of Lemongrass *(Cymbopogon citratus)*

Lemongrass *(Cymbopogon citratus)* is said to be indigenous to India where it has been cultivated for its oil since 1888. Lemongrass *(Cymbopogon citratus)* has been used in traditional Indian Medicine for a long time to treat fever and disease. In addition, in traditional Chinese Medicine, Lemongrass *(Cymbopogon citratus)* is used to treat rheumatism, headaches, colds and stomach pain.

Over the years, Lemongrass *(Cymbopogon citratus)* also has fast turned out to be the most wanted plant for the American gardeners and this is attributed to the increasing popularity of Thai culinary in the United States. The aromatic Lemongrass *(Cymbopogon citratus)* is considered to be of multi-purpose use in the kitchen as it is used in teas, drinks, herbal medications and the soups and delicacies originated in the Eastern region of the world and now popular all over. In fact, the worth of this aromatic and cosmetic plant was known to the ancient Greeks, Romans and Egyptians.

2.3.2 Chemical Constituents of Lemongrass *(Cymbopogon citratus)*

Fresh Lemongrass *(Cymbopogon citratus)* contains approximately 0.4% volatile oil. The oil contains 65% to 85% citral, a mixture of 2 geometric isomers, geraniol and neral. Related compounds geraniol, geranic acid, and nerolic acid have also been identified. Other compounds found in the oil include myrcene (12% to 25%), diterpenes, methylheptenone, citronellol, linalol, farnesol, other alcohols, aldehydes, linalool, terpineol, and more than a dozen other minor fragrant components. The large amounts of citral and geraniol in Lemongrass *(Cymbopogon citratus)* are lemon-scented and rose-scented respectively. Nonvolatile components of C. citratus consist of luteolins, homo-orientin, chlorogenic acid, caffeic acid, p -coumaric acid, fructose, sucrose, octacosanol, and others. Flavonoids luteolin and 6-C-glucoside have also been isolated. Lemongrass *(Cymbopogon citratus)* also includes nutritious calcium, iron, magnesium, manganese, phosphorus, potassium, selenium and zinc. Geographical variations in the chemical constituents have been noted.

Lemongrass *(Cymbopogon citratus)* oil has a lemony, sweet smell and is dark yellow to amber and reddish in color, with a watery viscosity. Lemongrass *(Cymbopogon citratus)* essential oil is characterized by a high content of citral (composed of neral and geranial isomers), which is used as a raw material for the production of ionone, vitamin A and beta-carotene (Mine *et al.*, 1994).

2.3.3 Application of Lemongrass *(Cymbopogon citratus)*

Lemongrass *(Cymbopogon citratus)* is cultivated in tropical regions around the world, in Guatemala, India, the People's Republic of China, Paraguay, England, Sri Lanka, and other parts of Indo-China region, Africa, Central America, and South America, for its oil used in perfumes, as a culinary flavoring and as a medicine.

2.3.3.1 Health Benefits

Lemongrass *(Cymbopogon citratus)* essential oil is analgesic, anti-microbial, antiseptic, astringent, bactericidal, carminative, deodorant, insecticidal, sedative, nervine and a tonic (Mohammad *et al*, 2009). Therefore, it gives various beneficial for a number of health problems (Organic Herbs Medicine Cabinet, 2008)

- Stress: As a mild sedative, Lemongrass's myrcene is an effective relaxant that acts as central nervous system depressant and helps people under stress and hypertension.
- Fever: Lemongrass is an aromatic and cooling herb that is used to increase perspiration and relieve fevers and help treat minor, feverish illnesses.
- Urination: It also acts as a diuretic and helps promote urination and relieves retained water.
- Digestion: Citral help the gastrointestinal tract and ease indigestion, flatulence and stomach discomforts by relieve digestive disturbances and intestinal irritations.
- Infections: As an effective antifungal and antimicrobial, Lemongrass is believed to dispel bacterial infections and has been used to treat internal parasites.
- Colds: To treat colds, sore throats and flu (especially with headaches and fevers) and is reputed to reduce and slow the discharge of mucus in respiratory conditions, due in part to its astringent properties.
- Mutagenic : Lemongrass may possess anti-mutagenic properties

- Cholesterol: Rich in geraniol and citral, Lemongrass may contribute to lowering serum cholesterol by interfering with an enzyme reaction and inhibiting the formation of cholesterol from simpler fats
- Insect repellent: Used externally, the herb is an effective treatment for lice, ringworm, athlete's foot and scabies.
- Skin: A tonic and supplement that is believed to be of great benefit to the skin and nails and is often used by herbalists to help clear blemishes and maintain balanced skin tone.

2.3.3.2 Toxicology

Lemongrass *(Cymbopogon citratus)* essential oil is considered to be non-toxic; however, it may cause some skin sensitization in individuals with sensitive or damaged skin or in those with allergies. For this reason, Lemongrass *(Cymbopogon citratus)* essential oil should be used with care and should not be used on young children. As is the case when using any essential oils, professional advice should be sought for individual concerns and by those inexperienced in the use of essential oils. Besides, Lemongrass *(Cymbopogon citratus)* should not be used in pregnancy because of uterine and menstrual flow stimulation (Mohammad *et al.*, 2009).

2.4 SEPARATION PROCESS

In chemistry and chemical engineering, a separation process is used to transform a mixture of substances into two or more distinct products. The separated products could differ in chemical properties or some physical property, such as size, or crystal modification or other separation into different components. Separation applications in the field of chemical engineering are very important. A good example is that of crude oil which is a mixture of various hydrocarbons and is valuable in this natural form. Separation processes can essentially be termed as mass transfer processes.

The mixture at hand could exist as a combination of any two or more states such as solid-solid, solid-liquid, solid-gas, liquid-liquid, liquid-gas, gas-gas and solid-liquid-gas mixture. Depending on the raw mixture, various processes can be employed to separate the mixtures. Many times two or more of these processes have to be used in combination to obtain the desired separation.

2.4.1 Separation of a Mixture by Extraction

Separation of a mixture by extraction is a process where the transfer of a solute from one phase to another. The purpose of separation of mixture by extraction is to isolate or to concentrate components from a mixture and also to separate components from other species that would interfere in the analysis. In extracting method, solvent should be similar to the solute. Mixtures can be separated by some methods, those are as follows:

- Distillation
- Chromatography
- Extraction
- Crystallization

The extraction process can be classified based on combination of phases (solid, liquid, gas, supercritical fluid). For solid – liquid, this extraction is useful for the isolation and purification of naturally occurring sources. While liquid – liquid is a more common method depending on solubility properties of components. These are the solvents used in separation of a mixture by extraction (Kosar *et al*., 2005):

- Organic solvents which are less dense than water : Diethyl ether, Toluene, Hexane

- Organic solvents which are denser than water: Dichloromethane, Chloroform and Carbon tetrachloride

2.4.2 Extraction of Essential Oil

Essential oils occur in many different parts of plants such as roots (vetiver), bark (cinnamon), heartwood (sandalwood), leaves (bay), herb (peppermint), seeds (nutmeg), flowers (cananga and jasmine). These essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method for extraction is steam distillation, but as technological advances is made more efficient and economical methods being developed (FAO Agricultural Services).

2.4.3 Distillation of Essential Oil

The essential oil of a plant consists of many compounds which generally boil between 150° to 300° C. If attempts are made to remove these compounds by dry distillation many will decompose and the oil will be ruined. However, the compounds are steam volatile and can be distilled out of the vegetal materials at around 100° C. Distillation converts the volatile liquid (the essential oils) into a vapor and then condenses the vapor back into a liquid. It is the most popular, and cost effective method in use today in producing essential oils.

The downside of distillation is the fact that heat is used in this extraction method, which makes it totally unacceptable for use on very fragile material, or where the oils are extracted with great difficulty. When this method of extraction is applied, great care has to be taken with the temperature and length of exposure of the heat to prevent damage to the oils.

Preparation of material for distillation varies with the material to be distilled. Some material must be distilled immediately after harvesting, whereas others can be (and are best) stored for a day or two before distilling and finally there are materials which can be stored indefinitely before distillation. In general, flowers should be distilled immediately, whereas herbaceous material often benefits from wilting for one or two days before distillation. Woody materials may need to be ground and/or soaked before distillation.

There are three basic types of essential oil distillation. The description of those distillation processes is further detailed in Table 2.3.

Table 2.3: Description of distillation processes in extraction of essential oil

Types	Process Desription	Advantages	Disadvantages
Hydrodistillation	-The steam is fed in from the top onto the botanical material -The condensation of the oil containing steam mixture occurs below the area in which the botanical material is held in place by a grill.	• less steam is used-shorter processing time • higher oil yield	• heat is difficult to control • possibility exists for local overheating and "burning" of the charge which can lead to a poorer quality oil
Water Distillation	-Botanic material is completely immersed in water and the still is brought to the boil -When the condensed material cools down, the water and essential oil is separated and the oil decanted to be used as essential oil.	- can be done at reduced pressure (under vacuum) - protects the oils so extracted to a certain degree since the surrounding water acts as a barrier to prevent it from overheating	• not suitable for botanical material that contains high amounts of esters
Steam Distillation	-The plant material is placed into a still (very similar to a pressure cooker)where pressurized steam passes through the plant material. -The heat from the steam causes globules of oil in	• relatively rapid, therefore charging and emptying the still is much faster • energy consumption is	• time consuming • high temperatures used may alter compounds.

	the plant to burst and the oil then evaporates -The essential oil vapor and the steam then pass out the top of the still into a water cooled pipe where the vapors are condensed back to liquids. The essential oil separates from the water and floats to the top.	lower • rapid distillation is also less likely to damage those oils which contain reactive compounds (esters)	

2.5 AN INTRODUCTION OF MICROWAVE-ASSISTED HYDRODISTILLATION (MAHD) AND OPERATION

Microwave assisted hydrodistillation (MAHD,) is another method of extraction in this area. This is an advanced hydrodistillation method based on the use of a microwave oven. It has an incontestable place in analytical and organic laboratory practices as a very effective and non-polluting method of activation. There are numerous examples of applications of this technology in sample digestion, organic synthesis, analytical chemistry and the food industry. Microwave energy, with a frequency of 2.45 GHz, is well known to have a significant effect on the rates of a variety of processes. The number of reported applications, especially in the food industry, is increasing rapidly. The main reason for this increased interest lies in the much shorter operation times achievable. Microwave–assisted extraction of natural compounds is also an alternative to conventional techniques. Essential oils are among the products which have been extracted efficiently from a variety of matrices by this method, and many microwave-assisted essential oil extractions from several plants and subsequent product analyses have been reported (Hong-Wu *et al.,* 2007).

The efficiency of MAHD is strongly dependent on the dielectric constant of water and the matrix. MAHD causes the rapid delivery of energy to the total volume of solvent or sample leading to a rapid rise in the temperature. Heat is originated through the molecular motions within the polar components or ionic species. That is, the rise in temperature within the plant cells is similar to that occurring outside the cells. Once the pressure within the glands reaches above certain level, the external cell walls break apart and as a consequence the essential oils are released to the environment. From this point on, the essential oils are carried away by water vapor, liquefied in the condenser on the top of the main apparatus and collected in the receiving flask. Because of different densities and also due to their immiscibility, water and essential oils are separated from each other and the excess water is refluxed to the extraction vessel (*Rezvanpanah et al., 2008*).

2.6 ANALYSIS

In order to examine the quality of an essential oil, a fine analysis on its composition need to be carried out. This is because; the types and amount of composition of particular components can reveal the characteristics of an essential oil. There are a number of analysis methods available in industry to carry out such examination.

2.6.1 Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography mass spectrometry (GC/MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identified and quantified. This makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds found in environmental materials. This included the analysis of essential oil from plants.

In order for a compound to be analyzed by GC/MS it must be sufficiently volatile and thermally stable. In addition, functionalized compounds may require chemical modification, prior to analysis, to eliminate undesirable adsorption effects that would otherwise affect the quality of the data obtained. Samples are usually analyzed as organic solutions consequently materials of interest such as soils,sediments, and tissues need to be solvent extracted and the extract subjected to various 'wet chemical' techniques before GC/MS analysis is possible.

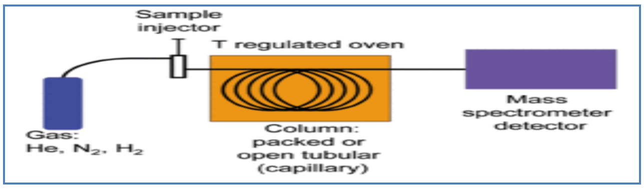


Figure 2.4: Schematic diagram of GC-MS

Source: Shila *et al.***,** 2011

2.6.2 Principle of Process of GC-MS

The GC-MS is composed of two major building blocks which are gas chromatograph and the mass spectrometer where the GC component will separates chemical mixture while MS component act as an identifier of components at a molecular level. The gas chromatograph utilizes a capillary column which depends on the column's dimensions in term of length, diameter, and film thickness and as well as the phase properties. The GC works on the principle that a mixture will separate, as the sample travels the length of the column, into individual substances when heated which is based on its chemical properties. The heated gases are carried through a column with an inert gas (such as helium). The molecules take different amounts of time which called as retention time to come out of or elute from the gas chromatograph. As the separated substances emerge from the column opening, they flow into the Mass Spectrometry and it will identify compounds by the mass of the analyte molecule. The mass spectrometer does this by breaking each molecule into ionized fragments. A “library” of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector.

CHAPTER 3

METHODOLOGY

3.1 PRETREATMENT

3.1.1 Ginger (*Zingiber Officinale* Roscoe)

Ginger (*Zingiber Officinale* Roscoe) rhizome was purchased from the supplier in Kuantan, Pahang which sold as fresh ginger in prices per kilo. The fresh ginger was then being sliced to an average thickness. After that, the sliced ginger rhizomes are dried in an open air for few days until it reached 90% dryness. The percent of dryness of the ginger can be determined by the following formula:

$$\text{Dryness (\%)} = \frac{|\text{Initial weight of sample (g)} - \text{Current weight of sample (g)}|}{\text{Initial weight (g)}} \times 100 \qquad (3.1)$$

Those dried ginger was then being grinded into small particles to increase the surface area that expose for distillation. In the extraction process, the rate of extraction increase when the surface area of materials increased. The grinded ginger rhizome was kept in a dark and dry box.

Before run the experiment 25g of grinded ginger was soaked for two hours into 200ml of distilled water for the ratio water to raw material of 8:1. Where else for ratio of 10:1 and 12:1, 25g of grinded ginger were soaked into 250ml and 300ml of distilled water, respectively. The summary of raw material preparation for Ginger (*Zingiber Officinale* Roscoe) is shown below:

1) Fresh Ginger (*Zingiber Officinale* Roscoe)

2) Fresh Ginger (*Zingiber Officinale* Roscoe) sliced into average thickness

3) Sliced Ginger (*Zingiber Officinale* Roscoe) dried until it reach 90% dryness

4) Dried Ginger (*Zingiber Officinale* Roscoe) grinded into small particles

Figure 3.1: Summary of raw material preparation for Ginger (*Zingiber Officinale* Roscoe)

3.1.2 Lemongrass (*Cymbopogon citratus*)

Lemongrass (*Cymbopogon citratus*) *leaves* was obtained from the residents around Gambang housing area in Kuantan, Pahang. The leaves were first chopped into small pieces to increase the surface area expose for distillation. In the process extraction of essential oil, the rate of extraction increase when the surface area of materials increased.

The chopped lemongrass leaves were then kept in a dark and cold refrigerator until it used for the experiments. Before run the experiment 50g of chopped lemongrass leaves was soaked for two hours into 400ml of distilled water for the ratio water to raw material of 8:1. Where else for ratio of 10:1 and 12:1, 50g of chopped lemongrass leaves were soaked into 500ml and 600ml of distilled water, respectively. The summary of raw material for Lemongrass (*Cymbopogon citratus*) is shown below:

1) Fresh Lemongrass *(Cymbopogon citratus)*

2) The Lemongrass *(Cymbopogon citratus)* leaves were chopped into small pieces

Figure 3.2: Summary of raw material preparation for Lemongrass *(Cymbopogon citratus)*

3.2 MICROWAVE-ASSISTED HYDRODISTILLATION

Extraction of essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass (*Cymbopogon citratus*) were conducted by using Microwave-Assisted Hydrodistillation method. In that case, a domestic microwave oven (Samsung, 250v-50Hz, maximum: 800 watts) was modified for the distillation. A round bottom flask was set up within the microwave oven cavity. Then the soaked raw material, with its distilled water, was transferred into the flask and the experiment were run based on the parameters condition. For Lemongrass (*Cymbopogon citratus*), the soaked solution were grounded before transferred into the flask. A hydrodistillation set which had been placed outside the oven was used to collect the extracted essential oils based on the extraction time. However this period of time only started after the mixture of water and plant material achieved their boiling point as it produced the first drop of condensed vapor in condenser. This called as induction time.

3.3 OPTIMIZATION OF OIL EXTRACTION

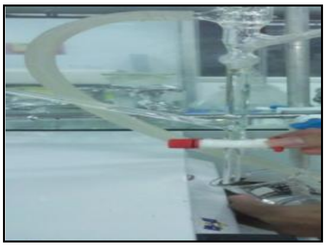
In order to optimize the extraction operating conditions for achieving maximum oil yield, the distillation was conducted at three unlike parameters conditions which were at different water to raw material ratio (v/w)(8:1, 10:1, 12:1), different extraction period (30min,60min,90min,120min,150min) and different operating power (200W, 250W). Following table and figure shows the overall condition for the extraction process and summary of extraction process, respectively.

Table 3.1: Overall condition of MAHD process

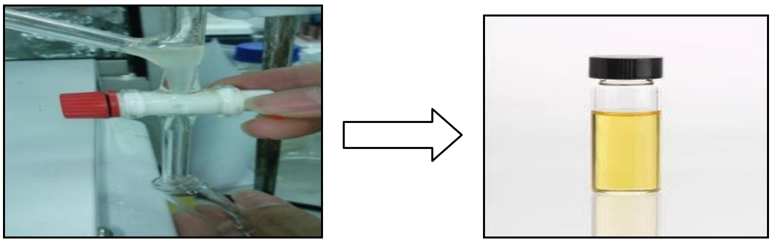
Frequency MHz	2450 (constant)
Temperature, ^{0}C	70 (constant)
Ratio (water : raw material), w/w	8:1, 10:1, 12:1
Extraction Time, min	30, 60, 90, 120, 150
Microwave Power, W	360, 540

1) The mixture of 25g of Ginger (*Zingiber Officinale* Roscoe) and its 200ml soaked distilled water (at ratio of 8:1) were transferred into 1L round bottom flask that set inside microwave oven cavity

2) Apparatus was setup for microwave-assisted hydrodistillation and the experiment was run at 250W for 30 minutes, which is after the induction period. The induction time was noted.



3) After 30 minutes, the microwave oven was turned off and the water fraction was discarded from the condensate (mixture of ginger essential oil and water) through the bottom of the condenser.

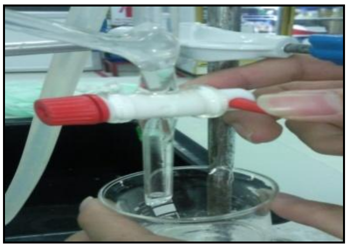


4) Oil sample was collected into a vial, dried and weighed. Then the vial was stored at 4^{0}C

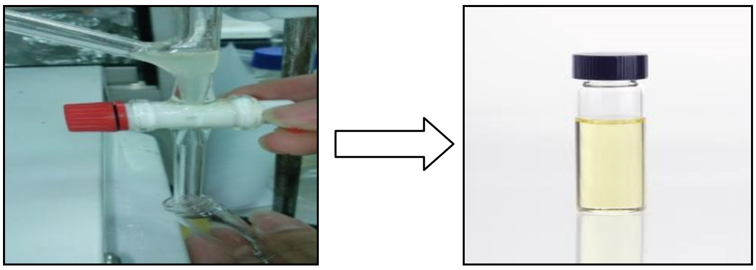
Figure 3.3: Summary of extraction of Ginger (*Zingiber Officinale* Roscoe) essential oil by Microwave-Assisted Hydrodistillation (at 250W/ ratio of 8:1/30min)

1) The mixture of 50g of Lemongrass *(Cymbopogon citratus)* and its 400ml soaked distilled water (at ratio of 8:1) were transferred into 1L round bottom flask that set inside microwave oven cavity

2) Apparatus was setup for microwave-assisted hydrodistillation and the experiment was run at 250W for 30 minutes, which is after the induction period. The induction time was noted.



3) After 30 minutes, the microwave oven was turned off and the water fraction was discarded from the condensate (mixture of lemongrass essential oil and water) through the bottom of the condenser.



4) Oil sample was collected into a vial, dried and weighed. Then the vial was stored at 4^0C

Figure 3.4: Summary of extraction of Lemongrass *(Cymbopogon citratus)* essential oil by Microwave-Assisted Hydrodistillation (at 250W/ ratio of 8:1/30min)

3.4 ANALYSIS OF SAMPLE

The condensate which consists of mixture of water and essential oil was collected in a vial with the water discarded. The extracted essential oils is dried over anhydrous sodium sulfate, weighed and stored in amber vials at 4 ^{0}c for the use of analysis.

3.4.1 Calculation of Yield of Extracts

The amount of yield obtained from the extraction was analyzed to evaluate the performance of MAHD in ginger oil and lemongrass oil extraction. As the result the yield of oil that obtained for every run was calculated by using the following equation:

$$\text{Yield of essential oil (\%)} = \frac{\text{amount of essential oil (g) obtained}}{\text{amount of raw materials (g) used}} \times 100\% \qquad (3.2)$$

3.4.2 Identification of Essential Oil Constituents

The extracted essential oil samples were analyzed by using Gas Chromatography Mass Spectrometry (GC-MS) Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and an Agilent Chem in order to identify their chemical constituents. This is an essential method to evaluate the quality of the oil samples.

First, for prepare the sample for GC-MS analysis, the extracted essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass (*Cymbopogon citratus*) were filtered using syringes and 0.25µm PTFE filters. Then the filtered samples were diluted with dichloromethane to a concentration of 2% by adding 20µL of pure essential oil to 980µL of dichloromethane to prepare 1mL samples. All the diluted samples were then put on the vortex mixer for 2 minutes to make the samples in homogeneous form.

The following operating parameters were used for Ginger (*Zingiber Officinale* Roscoe) essential oil sample: capillary GC column HP-5MS 5% phenylmethyl siloxane \ (30 x 0.25 mm i.d. x 0.25 mm film thickness), a carrier gas Helium (flow rate 1.2 mL min-1) and a split-less injection mode. Injector temperature is 250°C, Oven temperature will be set initially at 50°C, and then will be raised to 250°C at a 10°C min-1 rate till the end of analysis. The eluted analytes detected using (5973 network) mass selective detector and Electron Impact ionization (EID) will be carried out at 70 eV (Mahdi *et al*., 2010).

While, for Lemongrass (*Cymbopogon citratus*) essential oil sample, the operating parameters of GC-MS was as followed: system operating in EI mode (70 eV) , equipped with a split/splitness injector (280 ºC, split ratio 1:20) , using DB - 5 column (30 x 0.2 5 mm i.d x 0 . 2 5 mm) . The temperature program was 50ºC (5 min) rising to 300 ºC at rate of 5ºC/min. Injector and detector temperature was 280 º C .Helium was used as carrier gas at a flow rate 1 mL /min (Jigisha & Meghal, 2011) .

Essential oil constituents were identified by comparing retention times of the chromatogram peaks with those of reference compound run under identical conditions. Analytical result was obtained using the reference compounds based on the area ratio between target components and the reference compound.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION

This research was carried to study the efficiency of microwave-assisted hydrodistillation (MAHD) method on the extraction of essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)*.Therefore, the experiment was run at different parameters condition in order to indentify the most optimize condition for obtain a maximum yield of essential oil.

In that case, the parameters that been investigated were extraction time (30min, 60min ,90min,120min, 150min), microwave power(200W, 250W) and water to raw material ratio(8:1,10:1,12:1). Besides, the quality of the essential oil obtained through MAHD method also been analyzed using the GC-MS by identified its chemical constituents.

4.2 EFFECT OF EXTRACTION TIME ON YIELD

4.2.1 Ginger (*Zingiber Officinale* Roscoe)

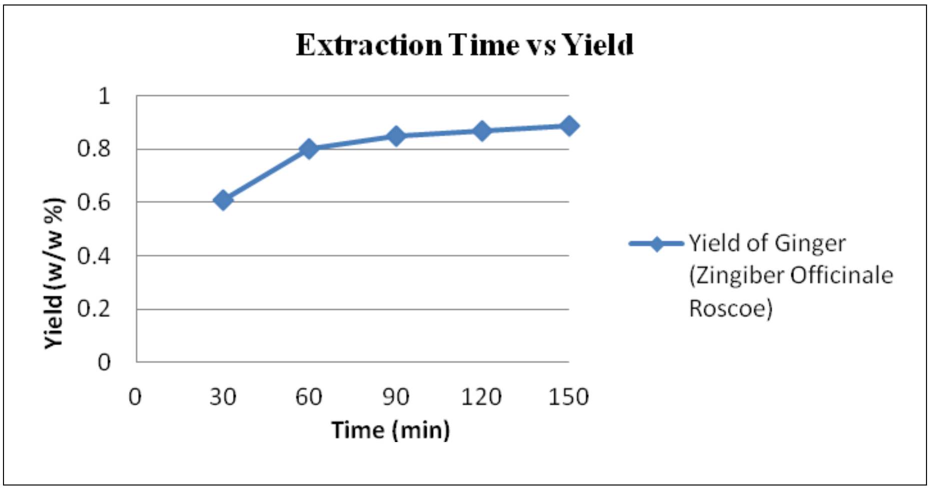


Figure 4.1: Extraction Yield of essential oil from Ginger (*Zingiber Officinale* Roscoe) at different extraction time by 250W in raw material to water ratio of 8:1

Figure 4.1 shows the yield of essential oil extracted from Ginger (*Zingiber Officinale* Roscoe) at different extraction time in a fix microwave power of 250W and water to raw material ratio of 8:1. From the graph, the amount of yield does not change significantly after 90 minutes. Most of the oil is extracted within 30 to 90 minutes which are with the yield (w/w) of 0.61 %, 0.80% and 0.85% respectively. Microwave assited hydrodistillation (MAHD) reach the highest yield (w/w) of 0.85% w/w when extraction time was 90 minutes. However, further increase in extraction time resulted in no improvement in the extraction performance. Similar observations were also reported for MAHD of Satureja hortensis and Satureja Montana by Rezvanpanah et al.,(2008). The extraction was fast at the beginning of the extraction but get slow gradually by time because when the raw material is exposed to the heat, the plant cell started to degrade and as a consequence the essential oil is released to the environment. However prolong the extraction time will cause over heat supplied to the plant material and this lead to the evaporation of the volatile component in the oil (*Rezvanpanah et al., 2008*). The results that show the effect of extraction time on yield of Ginger (*Zingiber Officinale* Roscoe) at 200W power and other water to raw material ratio (10:1 and 12:1) were given in the appendix A.

4.2.2 Lemongrass *(Cymbopogon citratus)*

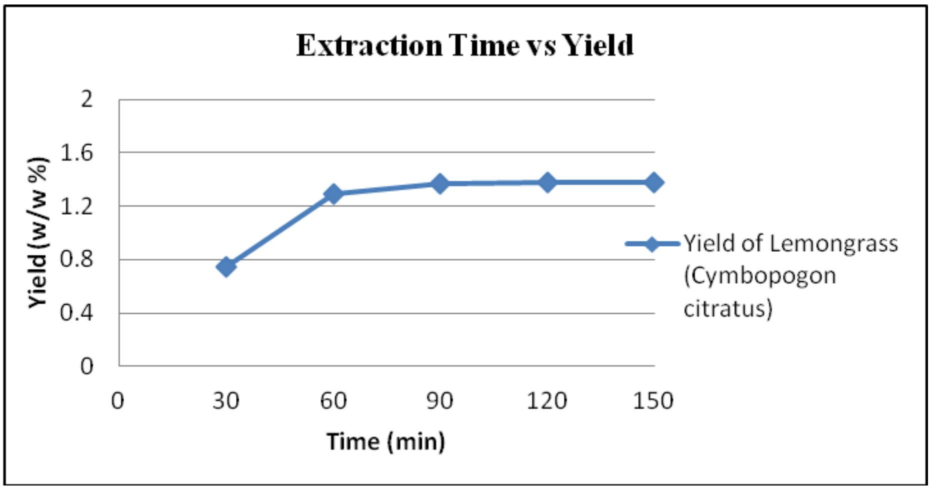


Figure 4.2: Extraction Yield of essential oil from Lemongrass *(Cymbopogon citratus)*at different extraction time by 250W in water to raw material ratio of 8:1

Figure 4.2 shows the yield of essential oil extracted from Lemongrass *(Cymbopogon citratus)* at different extraction time in a fix microwave power of 250W and water to raw material ratio of 8:1. From the graph, the amount of yield does not change significantly after 90 minutes. Most of the oil is extracted within 30 to 90 minutes which were with the yield (w/w) of 0.75%, 1.29% and 1.37% respectively. This define that the extraction was fast at the beginning of the extraction but get slow gradually by time. The same reason goes for Lemongrass *(Cymbopogon citratus)* as Ginger (*Zingiber Officinale* Roscoe) which is the vaporization of volatile oil due to overheat. The results that illustrate the effect of extraction time on yield of Lemongrass *(Cymbopogon citratus)* at 200W power and other water to raw material ratio (10:1 and 12:1) were given in the appendix A.

4.3 EFFECT OF MICROWAVE POWER ON YIELD

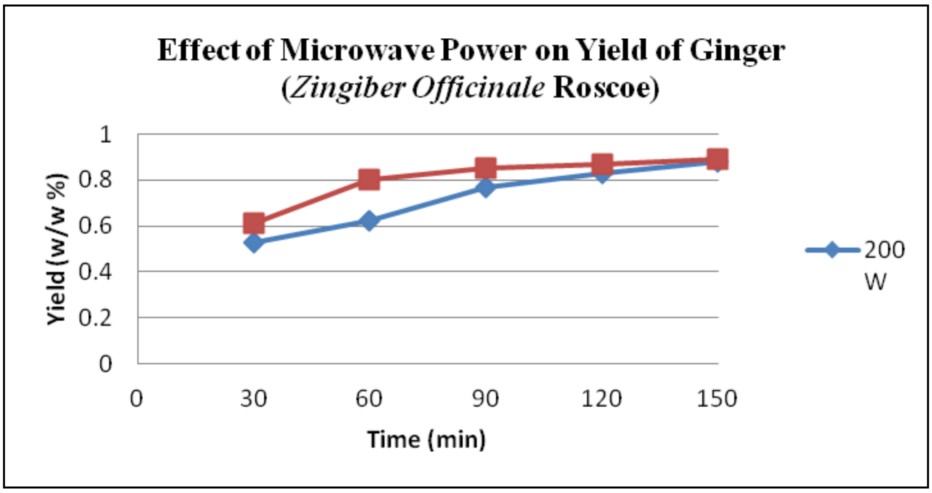


Figure 4.3: Variation of essential oil yield of Ginger (*Zingiber Officinale* Roscoe) at different microwave power level in water to raw material ratio of 8:1

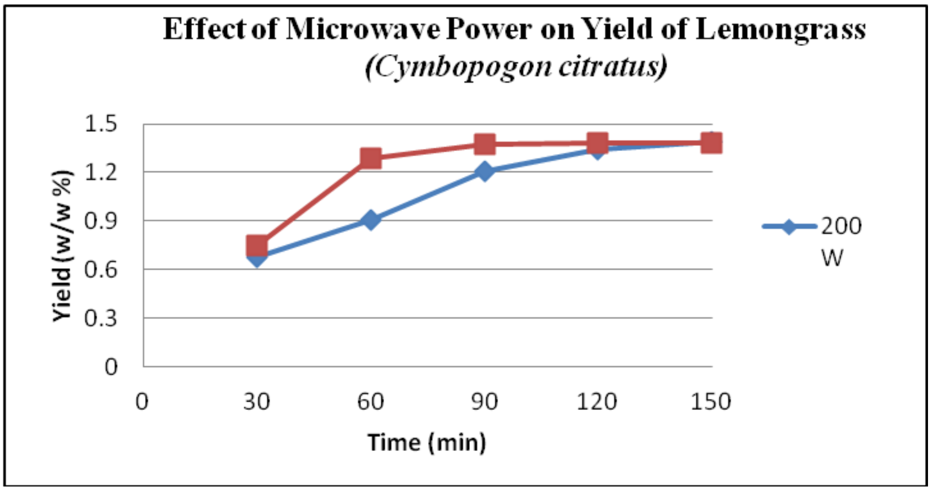


Figure 4.4: Variation of essential oil yield of Lemongrass *(Cymbopogon citratus)* at different microwave power level in water to raw material ratio of 8:1

The effect of microwave power on extraction yield of Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* at fix water to raw material ratio of 8:1 is shown in Figure 4.3 and Figure 4.4, respectively. From the graphs, it can be concluded that both of the plant materials give similar output pattern on the effect of microwave power.

Two microwave power level namely 200W and 250W were used in the extraction. In general, the extraction was improved by raising the microwave power from 200W to 250W. During short extraction time, 30 minutes to 90 minutes, yield of Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* was enhanced with microwave power increasing .The yield (w/w) of Ginger (*Zingiber Officinale* Roscoe)were 0.53% (30min), 0.62%(60min) and 0.77%(90min) at 200W whereas 0.61%(30min), 0.80%(60min) and 0.85%(90min) at 250W. While, yield(w/w) of Lemongrass *(Cymbopogon citratus)* were 0.68%,(30min), 0.91%(60 min) and 1.21%(90min) at 200W whereas 0.75%(30min), 1.29%(60min) and 1.37%(90min) at 250W. However, when the extraction solutions were heated long enough, 120 to 150 minutes, the yields under different power were similar.

As it clearly seen from the graphs, the initial extraction rate increased with increase in microwave power. This is doubtlessly due to the rapid generation of heat inside the immersed Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* with the absorption of microwave energy and the subsequent formation of a higher pressure gradient inside the plant material when subjected to higher microwave power levels. The essential oil has been released out of the glands quicker due to the increased gradient (Ferhat *et al*., 2006). The effect of microwave power on yield at water to raw material ratio of 10:1 and 12:1 for both plant materials were shown in the appendix A.

Another observation was the time to reach the boiling point of the mixture, which is known as the induction time, is shorter at higher microwave power level. This was proved by the induction time for Ginger (*Zingiber Officinale* Roscoe) which were 16 minutes(200W) and 8 minutes (250W)whereas for Lemongrass *(Cymbopogon citratus)*were 10 minutes (200W) and 7 minutes (250W) in fix water to raw material ratio of 8:1. Such result can be attributed to the more powerful effect of microwaves on water, a solvent with a high dielectric constant. However, the reduced in microwave power cause into longer induction time due to the lower density of waves at power level (Rezvanpanah *et al*., 2008).

4.4 EFFECT OF WATER TO RAW MATERIAL RATIO ON YIELD

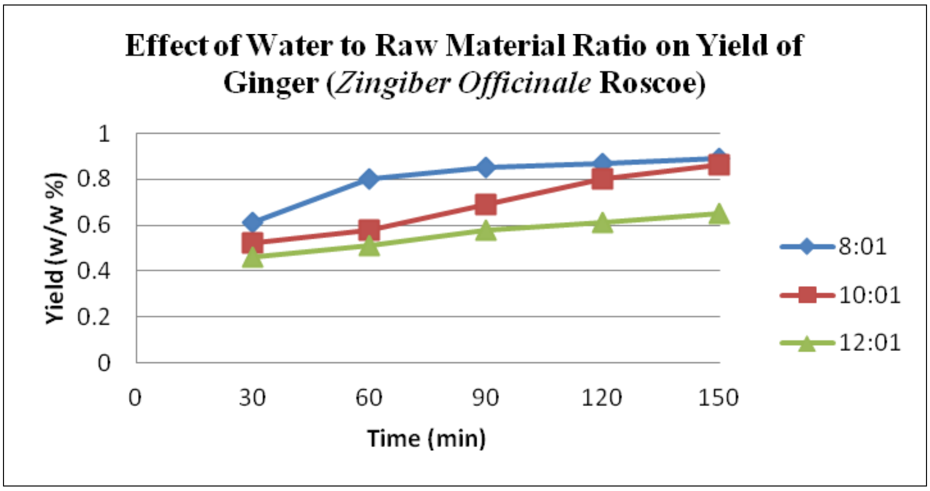


Figure 4.5: Variation of essential oil yield of Ginger (*Zingiber Officinale* Roscoe) in different water to raw material ratio at 250W

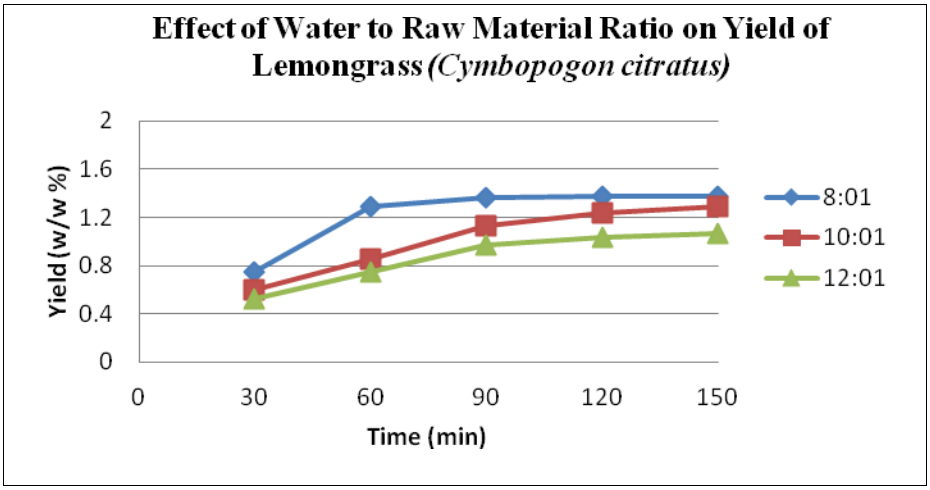


Figure 4.6: Variation of essential oil yield of Lemongrass *(Cymbopogon citratus)* in different water to raw material ratio at 250W

The effect of different water to raw material ratio on yield of Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* at fix power level of 250W is presented in Figure 4.5 and 4.6, respectively. The mass of plant material that used for the experiment is constant which were 25g for Ginger

(*Zingiber Officinale* Roscoe) and 50g Lemongrass (*Cymbopogon citratus).* On the other hand, the amount of distilled water that used as solvent is varied based on the ratio.

Both plant materials had illustrated similar output pattern on the variability of water to raw material ratio where the highest yield obtained at ratio of 8:1 followed by 10:1 and 12:1. The highest yield (w/w) at 8:1, 10:1 and 12:1 for Ginger (*Zingiber Officinale* Roscoe) were 0.89%, 0.86% and 0.65%, respectively and for Lemongrass *(Cymbopogon citratus)* were 1.38%, 1.29% and 1.07%, respectively. Therefore, it can be concluded that the yield from plant materials is increase when the amount of water as solvent is reduced. This was because the reduction of water content would minimize the degradation, trans-esterification or oxidation. Besides, presence of excess amount of water can cause excess thermal stress due to rapid heating of the solution on account of effective absorption of microwaves by water (Dhobi, Mandal and Hemalatha, 2009).

In addition, both of the graphs clearly demonstrated that there is no significant difference on yield after 90 minutes in ratio of 8:1. The reason is that the small amount of water in the system failed to withstand at high microwave intensity for a longer extraction time and started to evaporate. This cause the plant material burned in the flask. This could increase the maintenance cost in industrial level.

Generally, the main function of water in distillation is to prevent the raw material from being thermally degraded and also act as the carrier of essential oil during the evaporation before the condensation process took place. Conversely, by limiting the total quantity of water in a closed cycle operation, it is possible to obtain increased yield of essential oil which are more water soluble (Sefidkon, Abbasi, & Khaniki,2005) Therefore it is important to identify the optimum level of water in the distillation system to avoid the reverse act of the water. The results that illustrate the effect of water to raw material ratio on yield of Ginger (*Zingiber Officinale* Roscoe) and Lemongrass (*Cymbopogon citratus)* at 200W power were given in the appendix A.

Another observation in this area is the effect of water to raw material ratio on the induction time. Based on the result obtained, the induction time decreases when smaller amount of water applied as solvent because it will boil more faster compared to large amount of water. The result for both plant materials at 250W are illustrated in following graph:

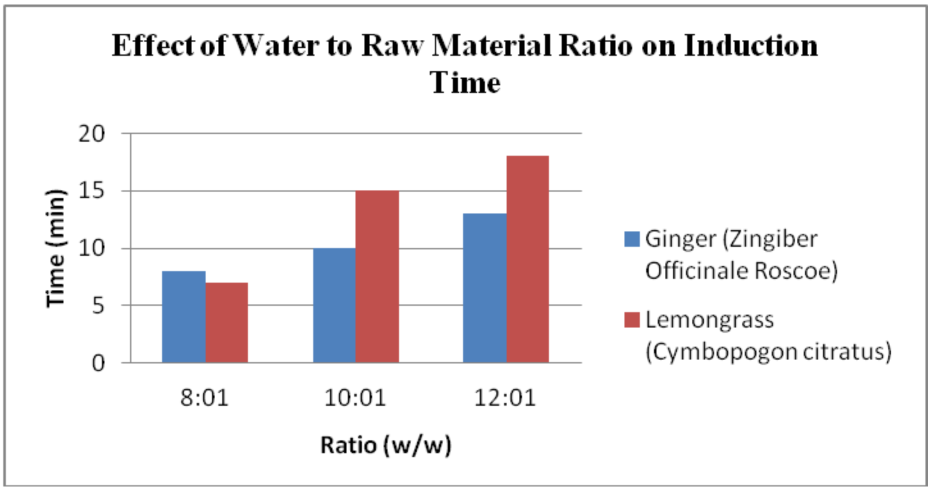


Figure 4.7: Effect of water to raw material ratio on the starting time of extraction in Ginger (*Zingiber Officinale* Roscoe) and Lemongrass (*Cymbopogon citratus)*at 250W

4.5 IDENTIFICATION AND QUANTIFICATION OF EXTRACTED ESSENTIAL OIL

In the analysis of GC-MS on Ginger (*Zingiber Officinale* Roscoe) and Lemongrass (*Cymbopogon citratus)*, there were total 35 components identified in essential oil of Ginger (*Zingiber Officinale* Roscoe) and 63 components identified in essentiall of (*Cymbopogon citratus).* The main components detected in the essential oil of Ginger (*Zingiber Officinale* Roscoe) were Borneol, β-Bisabolene, Cineole, α-Cedrene, α-Curcumene, β-Farnesene (E), β-Sesquihelladiene, β-Thujene and Zingiberene. Wherelse, the main components in the essential oil of Lemongrass (*Cymbopogon citratus)* were Citral, (Z) Citral, β-mycrene and β-Thujene. The dominant component in the essential oil of Ginger (*Zingiber Officinale* Roscoe) and Lemongrass (*Cymbopogon citratus)* were Zingiberene and Citral, respectively.

4.5.1 Effect of Extraction Time on the Chemical Constituents of Ginger (*Zingiber Officinale* Roscoe) Essential Oil

Table 4.1: Major chemical constituents of Ginger (*Zingiber Officinale* Roscoe) essential oil at different extraction time

Constituents	Relative Peak Area,% at Different Extraction Time				
	30min	**60 min**	**90 min**	**120 min**	**150 min**
Borneol	-	4.73	-	-	-
β-Bisabolene	9.98	-	9.72	-	10.41
Cineole	6.08	-	-	-	-
α-Cedrene	-	-	4.64	8.37	4.75
α-Curcumene	11.82	11.88	12.18	-	14.50
β-Farnesene (E)	-	8.80	-	-	-
β-Sesquihelladiene	11.41	10.62	11.19	14.43	13.02
β-Thujene	-	9.14	6.69	6.59	-
Zingiberene	29.76	22.80	24.53	49.81	24.80

The main chemical constituents identified from the essential oil of Ginger (*Zingiber Officinale* Roscoe) at different extraction time are presented in Table 4.1. Each extraction time gives different main constituents in different amount. The GC-MS chromatogram for these major constituents and other constituents were included in the appendix B.

The oil sample collected at 30 minutes of extraction time was gives five main components which were β-Bisabolene(9.98%), Cineole (6.08%), α-Curcumene (11.82%), β-Sesquihelladiene (11.41%) and Zingiberene(29.76%). The amount of each constituent within the essential oil in descending order: Zingiberene> α-Curcumene> β-Sesquihelladiene> β-Bisabolene> Cineole.

While, six major components were identified in the essential extracted at 60 minutes time which were Borneol (4.73%), α-Curcumene (11.88%), β-Farnesene (E) (8.80%), β-Sesquihelladiene (10.62%), β-Thujene (9,14%) and Zingiberene(22.80%). List of these constituent in the order of descending amount: Zingiberene> α-Curcumene >β-Sesquihelladiene> β-Thujene> β-Farnesene (E)> Borneol.

Beside, the study on the essential oil extracted at 90 minutes time gives six major components. They are β-Bisabolene (9.72%), α-Cedrene (4.64%), α-Curcumene (12.18%), β-Sesquihelladiene (11.19%), β-Thujene (6.69%) and Zingiberene(24.53%) which can be arrange in the order with descending amount as follows: Zingiberene> α-Curcumene> β-Sesquihelladiene> β-Bisabolene>β-Thujene> α-Cedrene.

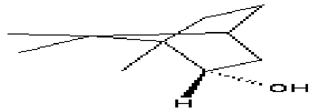
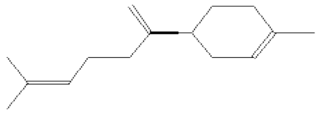
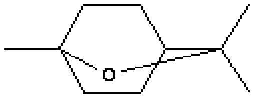
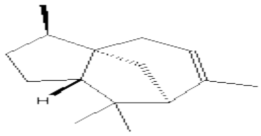
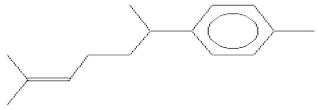
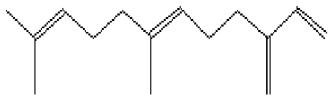
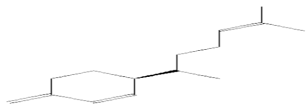
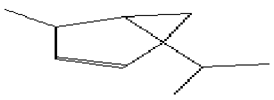
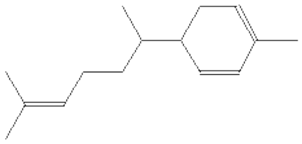
The 120 minutes extraction gives an essential with four major components which were α-Cedrene (8.37%), β-Sesquihelladiene (14.43%), β-Thujene (6.59%) and Zingiberene(49.81%). These can be arranging in the order with descending amount as follows: Zingiberene> β-Sesquihelladiene> α-Cedrene >β-Thujene.

Finally, the five main components that identified from the sample of oil extracted in 150 minutes time were β-Bisabolene (10.41%), α-Cedrene (4.75%), α-Curcumene (14.50%), β-Sesquihelladiene (13.02%) and Zingiberene(24.80%). If these components list in an order with descending amount: Zingiberene >α-Curcumene> β-Sesquihelladiene >α-Cedrene.

Overall, from the table it shows that Zingiberene is the key component at all extraction time with the highest amount in 120 minutes extraction, 49.81%. Two constituents that identified as major component in all essential oil samples were Zingiberene and β-Sesquihelladiene. Zingiberene is the key component of Ginger (*Zingiber Officinale* Roscoe) which gives it's the characteristic odor and flavor as well as the anti-fungal properties. Borneol and β-Farnesene (E) detected as major component only in the 60 minutes extraction and Cineole in 30 minutes extraction. When the samples being distilled, some kind of compound in it may have been oxidized, hydrolyzed or undergone other chemical reaction. This is what led to the differences in the composition and quantity of each constituent in the essential oil.

Among these nine major constituents, Borneol and Cineole are oxygenated monoterpenes wherelse the rest are sesquiterpene hydrocarbon. Sesquiterpene hydrocarbon has anti-inflammatory and anti-allergy properties. While, oxygenated monoterpenes have good antiseptic, anti-viral and anti-fungal properties. Therefore, ginger is highly suitable to be applied in medicinal field. The following table shows the chemical equation and structure of these major constituents:

Table 4.2: Chemical formula and structure of major constituents of Ginger (*Zingiber Officinale* Roscoe) essential oil

No	Constituent	Formula	Structure
1	Borneol	$C_4H_{18}O$	
2	β-Bisabolene	$C_{15}H_{24}$	
3	Cineole	$C_{10}H_{18}O$	
4	α-Cedrene	$C_{15}H_{24}$	
5	α-Curcumene	$C_{15}H_{22}$	
6	β-Farnesene (E)	$C_{15}H_{24}$	
7	β-Sesquihelladiene	$C_{15}H_{24}$	
8	β-Thujene	$C_{10}H_{16}$	
9	Zingiberene	$C_{15}H_{24}$	

4.5.2 Effect of Extraction Time on the Chemical Constituents of Lemongrass (*Cymbopogon citratus)* Essential Oil

Table 4.3: Major constituents of Lemongrass (*Cymbopogon citratus)* essential oil at different extraction time

Constituents	Relative Peak Area,% at Different Extraction Time				
	30min	**60 min**	**90 min**	**120 min**	**150 min**
Citral	46.95	44.24	48.16	50.45	65.80
β-Mycrene	-	4.23	-	-	-
β-Thujene	6.80	9.44	7.45	9.37	-
(Z)-Citral	34.20	32.96	34.64	33.67	38.15

The main chemical constituents identified from the essential oil of Lemongrass (*Cymbopogon citratus)* at different extraction time are presented in Table 4.3. Each extraction time gives different main constituents in different amount. The GC-MS chromatogram for these major constituents and other constituents were included in the appendix B.

The essential oil that extracted in 30 minutes contained 3 major constituents which were Citral (46.95%), (Z)-Citral(34.20%) and β-Thujene (6.80%).

While, there were four major components identified in the 60 minutes extracted oil which were Citral (44.24%), (Z)-Citral(32.96%), β-Thujene (9.44%) and β-Mycrene(4.23%).

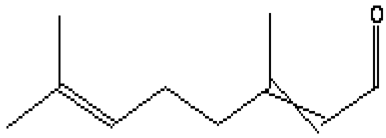
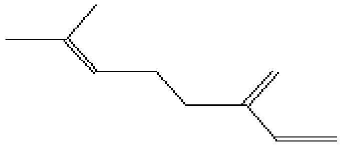
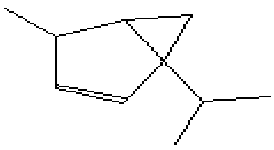
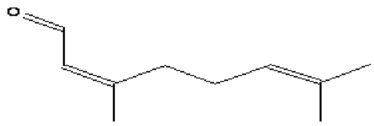
Besides, for 90 minutes of extraction, the essential oil contained 3 major components. They are Citral (48.16%), (Z)-Citral(34.64%) and β-Thujene (7.45%).

Citral (50.45%), (Z)-Citral(33.67%) and β-Thujene (9.37%) were the major components of essential oil that identified in 120 minutes extraction time sample.

There were only two major constituents determined in the sample of essential oil at 150 minutes extraction which were Citral (65.80%), (Z)-Citral(38.15%).

The amount of each constituent within the essential oil in descending order at all extraction time was same: Citral >(Z)-Citral>β-Thujene. From the table, it clearly shows that Citral is the key component of the Lemongrass (*Cymbopogon citratus)* essential oil which present in all the samples in a higher amount. The highest amount of Citral is obtained at extraction of 150 minutes which was 65.80%. Citral is the component of Lemongrass (*Cymbopogon citratus)* which give it lemony smell and anti-septic properties. The following table shows the chemical equation and structure of the major constituents in the essential oil of Lemongrass (*Cymbopogon citratus)*:

Table 4.4: Chemical Formula and Structure of Major Constituents of Lemongrass (*Cymbopogon citratus)* Essential Oil

No	Constituents	Formula	Structure
1	Citral	$C_{10}H_{16}O$	
2	β-Mycrene	$C_{10}H_{16}$	
3	β-Thujene	$C_{10}H_{16}$	
4	(Z)-Citral	$C_{10}H_{16}O$	

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This research was carried out to investigate the performance of microwave assisted hydrodistillation (MAHD) method in the extraction of essential oil from plant materials by examining their yield. Three main operating parameters affecting the microwave-assisted hydrodistillation method on extraction of essential oil from Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* were optimized based on the maximum yield extracted from the plant materials. The optimum condition for the lab scale MAHD extraction method was obtained under 250W microwave power for 90 minutes of extraction at water to raw material ratio of 8:1 which give the oil yield (w/w) of 0.85% for Ginger (*Zingiber Officinale* Roscoe) and 1.37% for Lemongrass *(Cymbopogon citratus)* . This optimum condition was applicable for the extraction of essential oil from both plant materials.

The major constituents found in the essential of Ginger (*Zingiber Officinale* Roscoe) were Borneol, β-Bisabolene, Cineole, α-Cedrene, α-Curcumene, β-Farnesene (E), β-Sesquihelladiene, β-Thujene and Zingiberene. Wherelse, the main components in the essential oil of Lemongrass (*Cymbopogon citratus)* were Citral, Geranic Acid, Geranyl Acetate, Linalool, Neric acid, (Z) Citral, β-mycrene and β-Thujene. The dominant component in the essential oil of Ginger (*Zingiber Officinale* Roscoe) and Lemongrass (*Cymbopogon citratus)* that detected in all samples of oils (30 minutes to 150 minutes) were Zingiberene and Citral, respectively. These two components are well known with their anti-microbial properties. As the result, Ginger (*Zingiber Officinale* Roscoe) and Lemongrass *(Cymbopogon citratus)* are being one of the major ingredients for most health and sanitary products. Therefore, it’s highly recommended to apply a method which is free of chemical in producing such essential oils due to its medicinal and consumption application. In that case MAHD is one of highly recommended method as it free of any chemical usage during its extraction process besides giving high yield at short extraction time without any affect on its constituents. This may proved MAHD as a cost effective method and expected would bring volume of worth in the essential oil production industry. Based on this study, it can be concluded that MAHD offered substantial advantage in this field.

5.2 RECOMMENDATIONS

To improve the essential oil production, there are three recommendations. First, throughout this study, the microwave assisted hydrodistillation method was run by modifying the domestic microwave oven. Therefore, it is recommended to scale up the process by enlarging the microwave heating system so that bigger flask can be used for examining large amount of plant material to obtain higher yield. Secondly, is height of the condensation unit should be increased as well as the condenser part. This is because the short condenser part leads to the quick movement of vapor through it which makes the vapor of essential oil doesn’t condensed fully before reach the bottom of the condenser unit. Thirdly is the size of the plant material. It strongly recommended grinding the plant material into smaller particles before run the experiment to increase its surface area. Increased in the surface area will give more efficient on the extraction process and yield.

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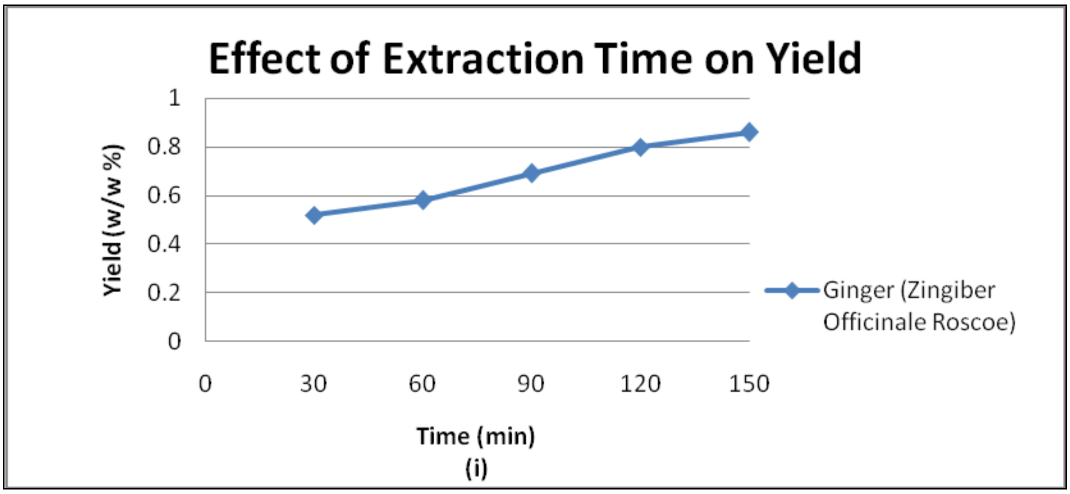
APPENDICES

APPENDIX A: EFFECT ON YIELD

Appendix A.1: Effect of Extraction Time on Yield of Ginger (*Zingiber Officinale* Roscoe)



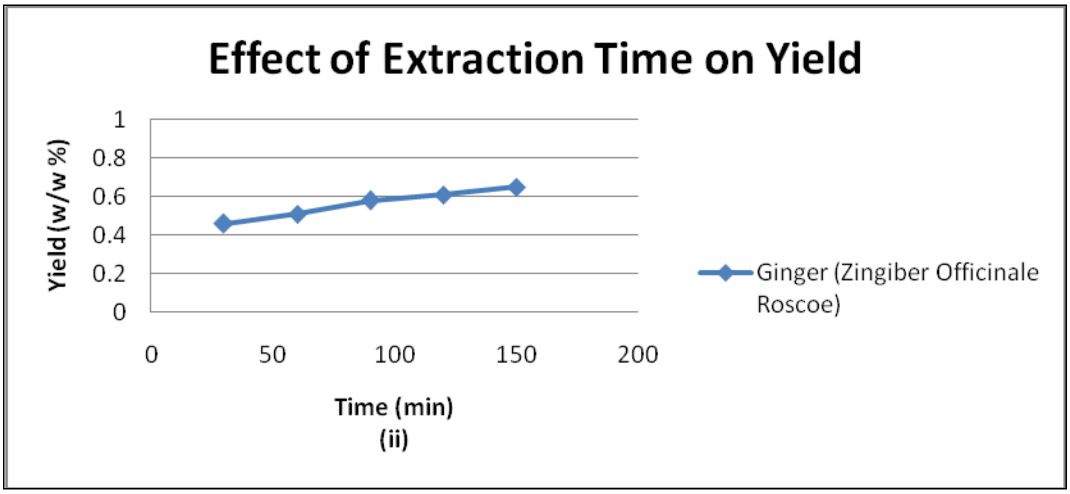


Figure A.1: Effect of Extraction Time on Yield at 250W under water to raw material ratio of i) 10:1and ii) 12:1

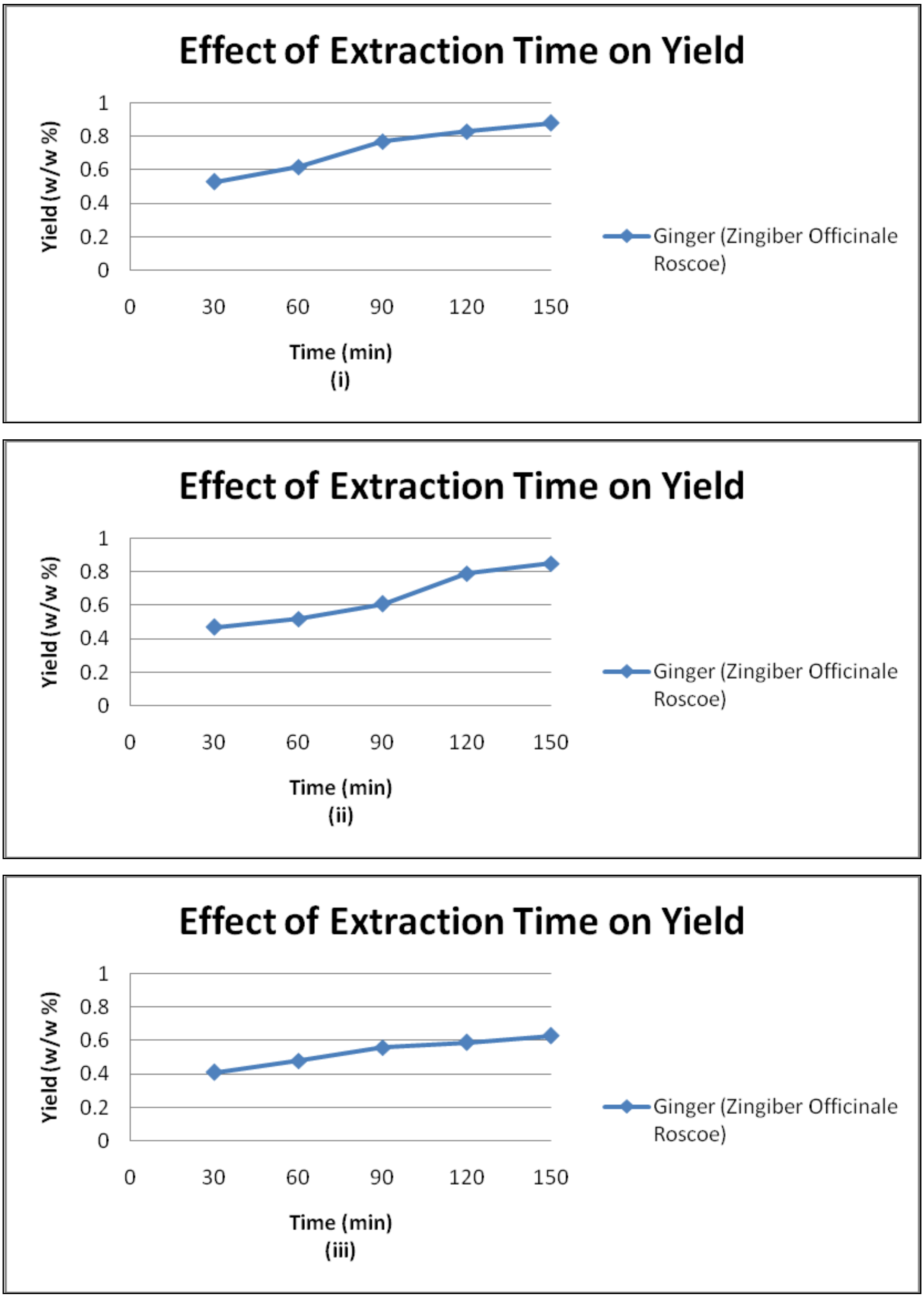
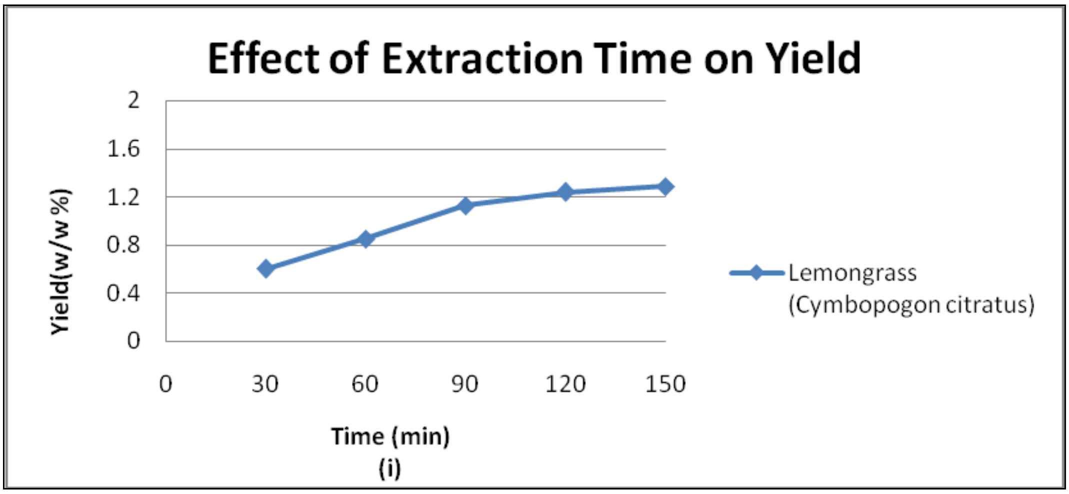


Figure A.2: Effect of Extraction Time on Yield at 200W under water to raw material ratio of i) 8:1, ii) 10:1 and iii) 12:1

Appendix A.2: Effect of Extraction Time on Yield of Lemongrass *(Cymbopogon citratus)*



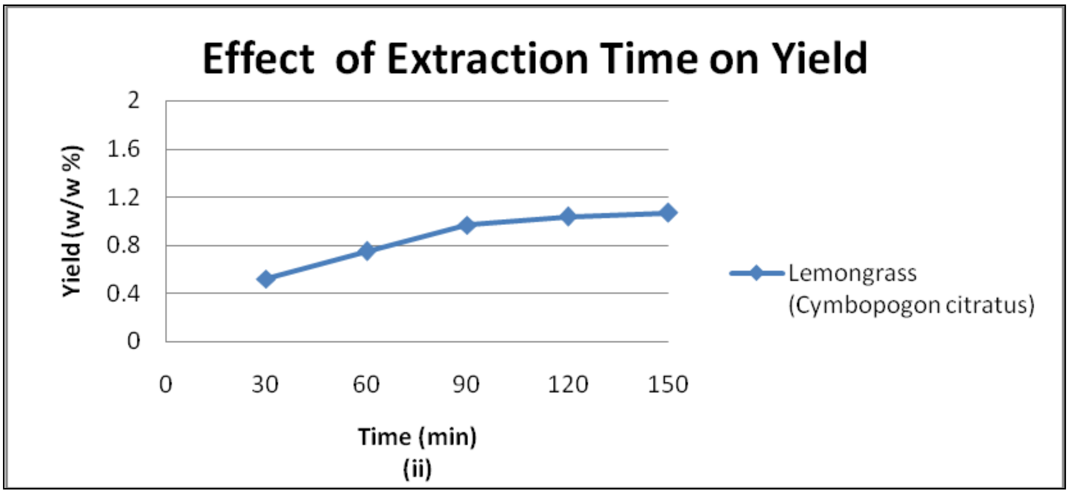
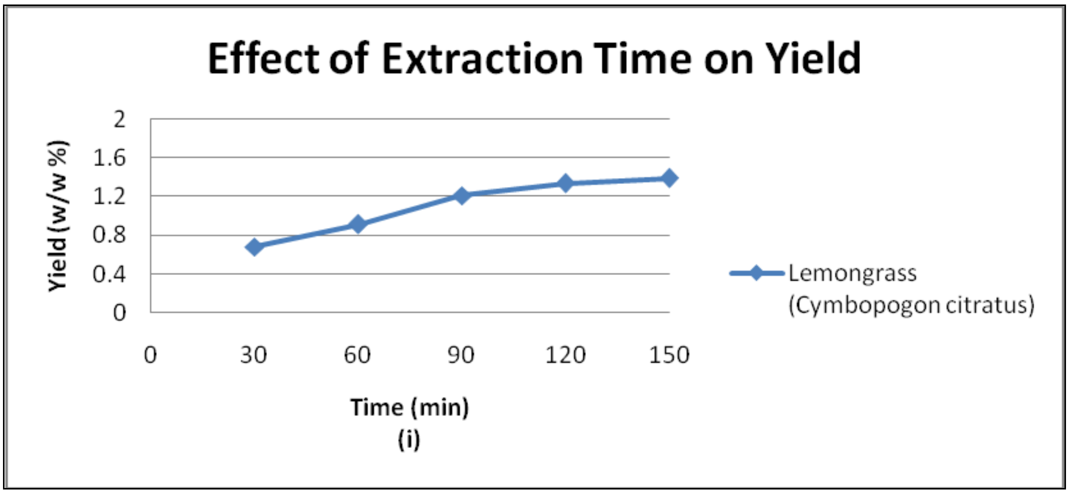
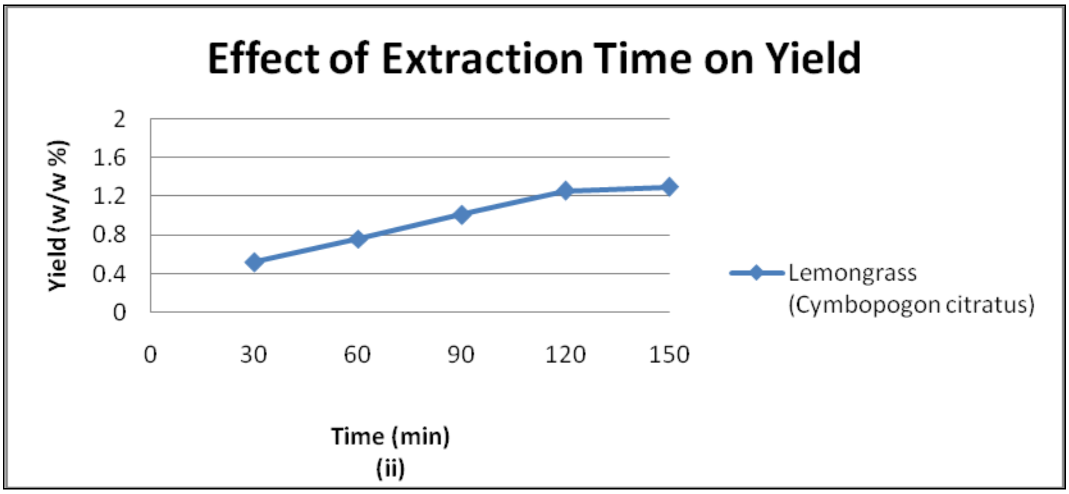


Figure A.3: Effect of Extraction Time on Yield at 200W under Water to Raw Material Ratio of i) 10:1 and ii) 12:1





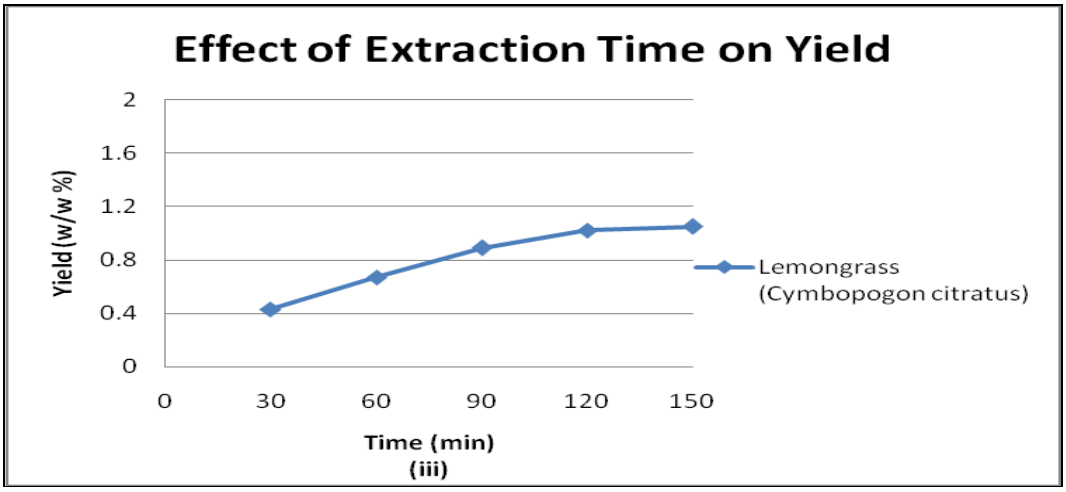
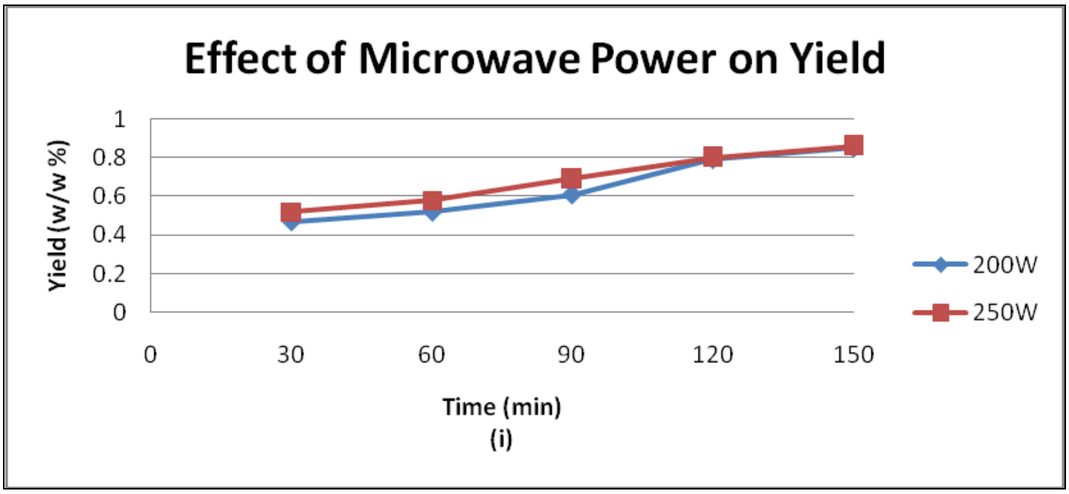


Figure A.4: Effect of Extraction Time on Yield at 200W under Water to Raw Material Ratio of i) 8:1, ii)10:1 and iii)12:1

Appendix A.3: Effect of Microwave Power on Yield of Ginger (*Zingiber Officinale* Roscoe)



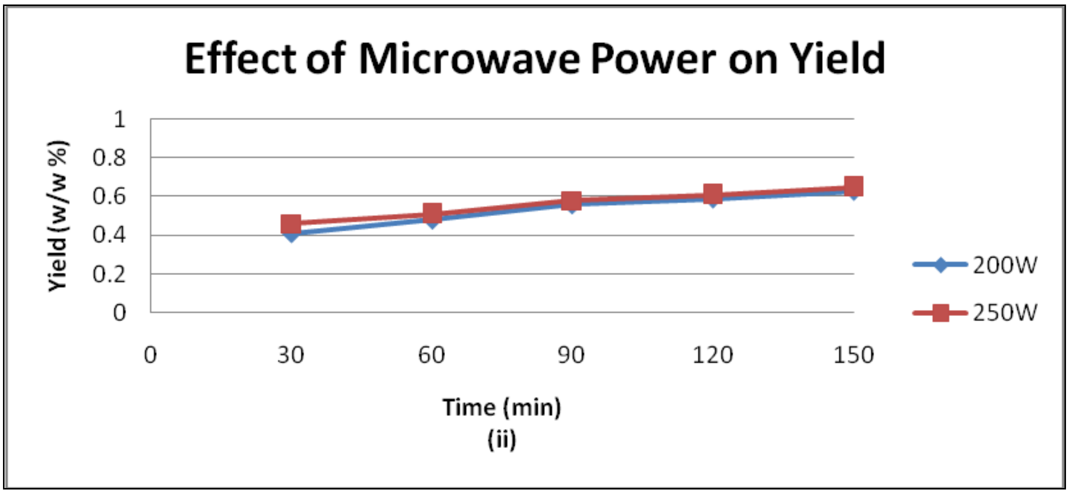


Figure A.5: Effect of Microwave Power on Yield under Water to Raw Material Ratio of i) 10:1 and ii) 12:1

Appendix A.4: Effect of Microwave Power on Yield of Lemongrass *(Cymbopogon citratus)*

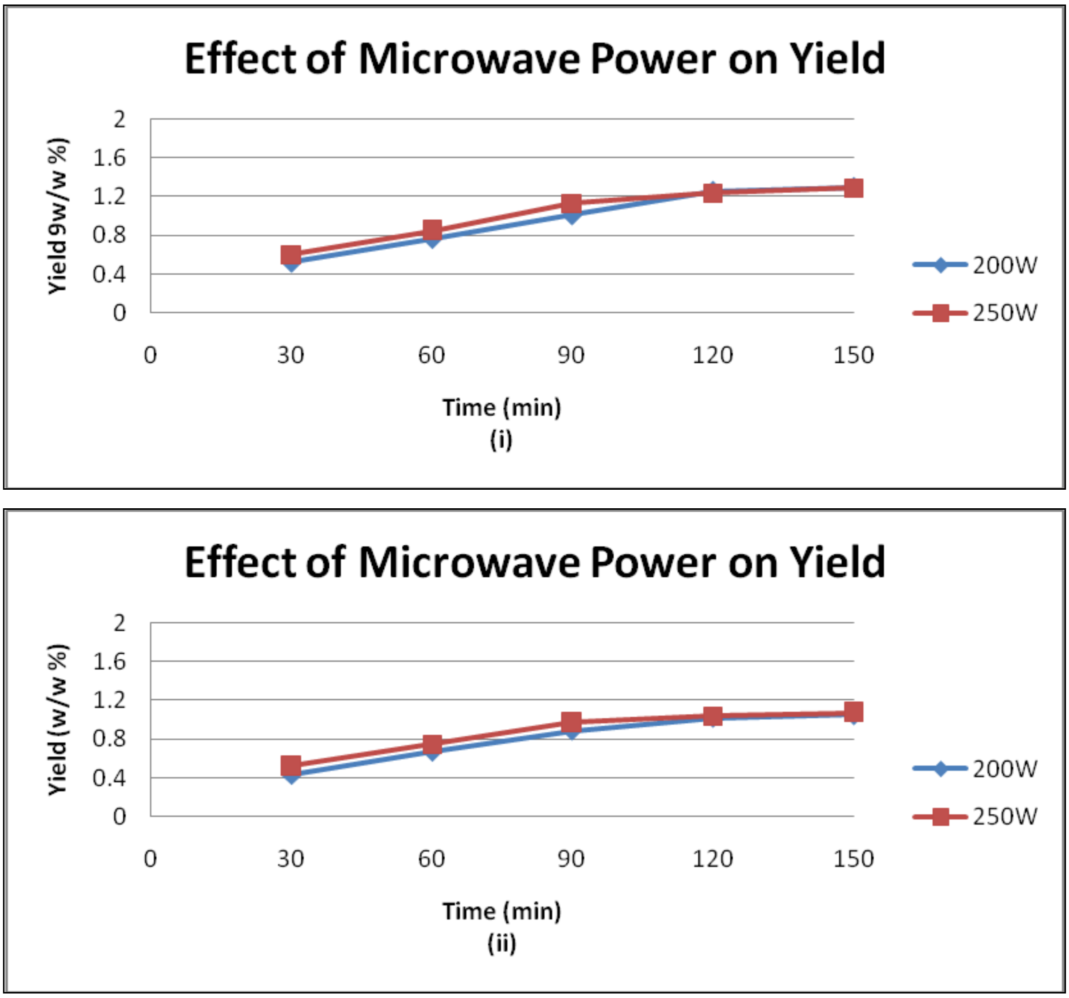


Figure A.6: Effect of Microwave Power on Yield under Water to Raw Material Ratio of i) 10:1 and ii) 12:1

Appendix A.5: Effect of Water to Raw Material Ratio on Yield of Ginger (*Zingiber Officinale* Roscoe)

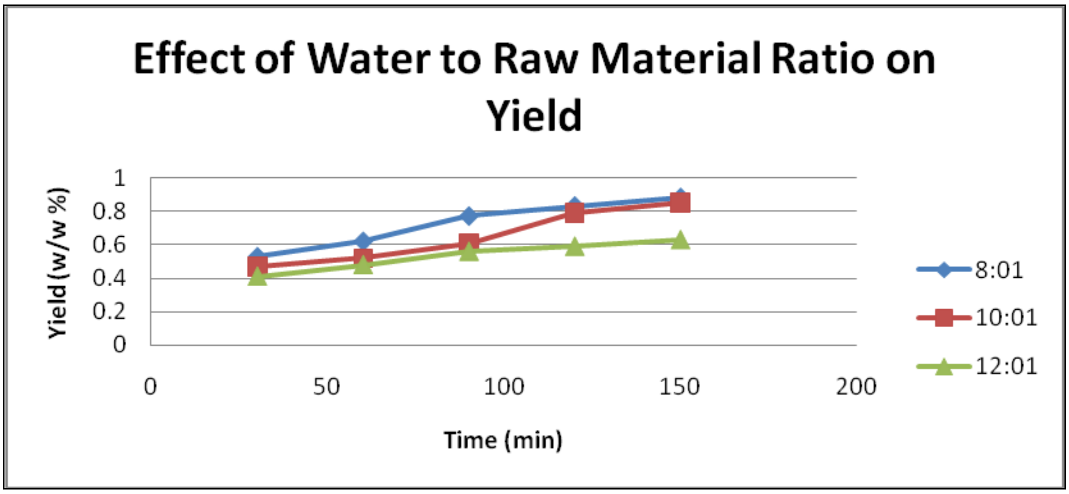


Figure A.7: Effect of Water to Raw Material Ratio on Yield at 200W

Appendix A.6: Effect of Water to Raw Material Ratio on Yield of Lemongrass *(Cymbopogon citratus)*

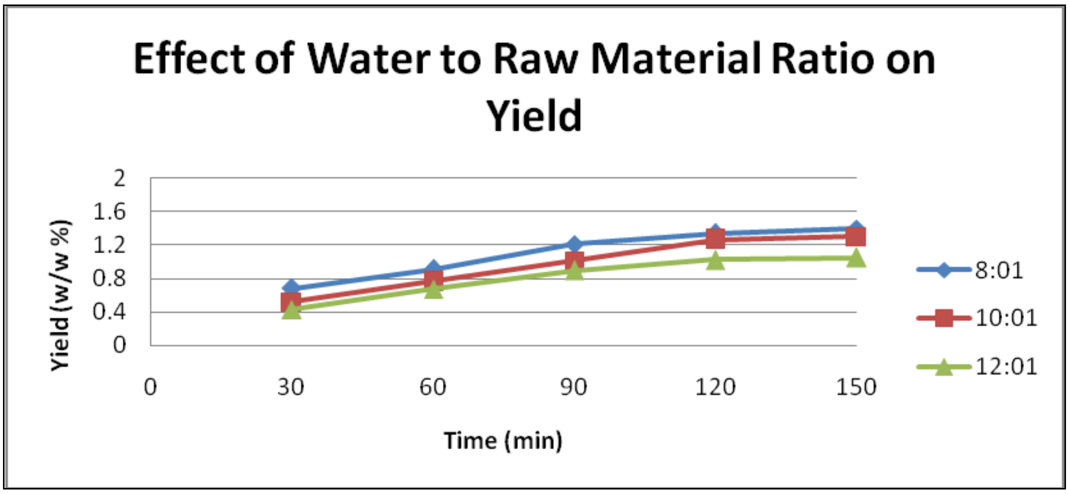
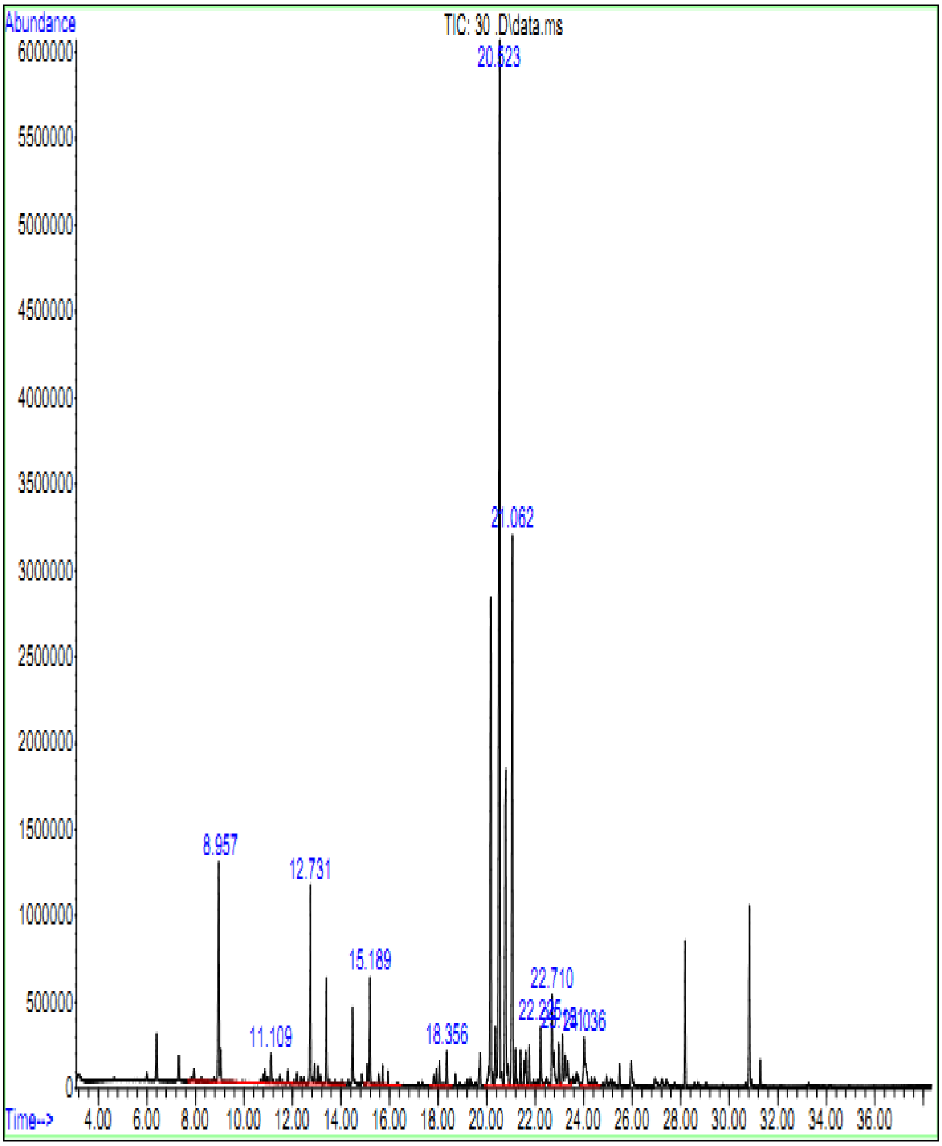


Figure A.8: Effect of Water to Raw Material Ration on Yield at 200

APPENDIX B: EFFECT ON CHEMICAL CONSTITUENTS OF EXTRACTED ESSENTIAL OIL

Appendix B1: Effect of Extraction Time on Chemical Constituents of Essential Oil of Ginger (*Zingiber Officinale* Roscoe)



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
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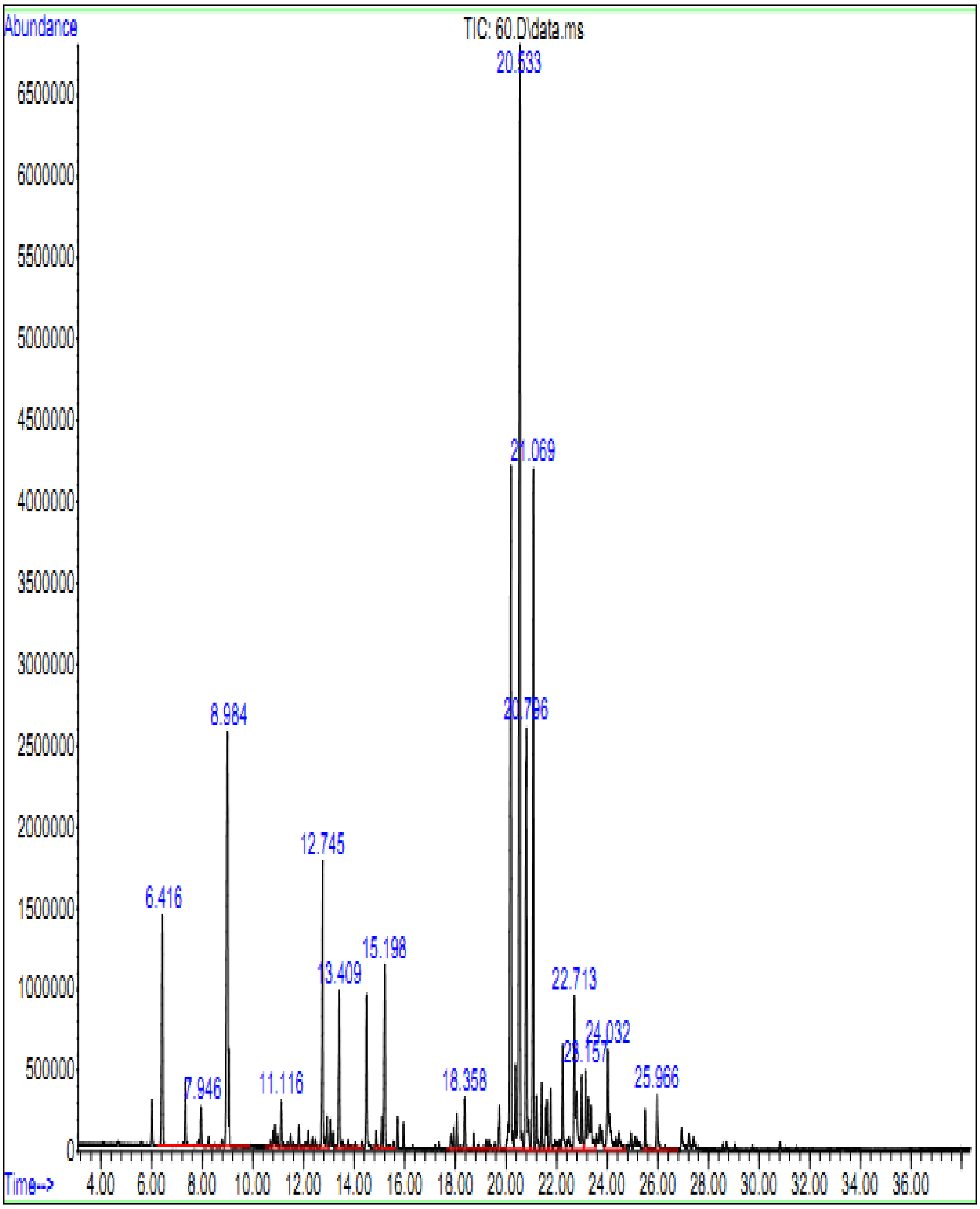
			Eucalyptol	25509	000470-82-6	98
			Eucalyptol	25508	000470-82-6	93
			Trifluoroacetyl-.alpha.-terpineol	91690	1000058-17-6	72
2	11.109	1.77	C:\Database\NIST05a.L			
			1,6-Octadien-3-ol, 3,7-dimethyl-	25643	000078-70-6	49
			1,6-Octadien-3-ol, 3,7-dimethyl-	25636	000078-70-6	46
			Linalyl isobutyrate	74304	000078-35-3	43
3	12.732	8.10	C:\Database\NIST05a.L			
			Borneol	25498	000507-70-0	91
			Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	25830	000464-45-9	91
			Borneol	25495	000507-70-0	91
4	15.189	3.22	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	96
			2,6-Octadienal, 3,7-dimethyl-, (E)	24151	000141-27-5	94
			2,6-Octadienal, 3,7-dimethyl-, (E)	24141	000141-27-5	94
5	18.356	1.79	C:\Database\NIST05a.L			
			Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	60003	000515-13-9	81
			Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	59911	110823-68-2	68
			Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	60001	000515-13-9	49
6	20.521	51.71	C:\Database\NIST05a.L			
			1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	59955	000495-60-3	90
			1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	59958	000495-60-3	90
			trans-(2-Chlorovinyl)trimethylsilane	14568	1000139-55-2	59
7	21.061	14.18	C:\Database\NIST05a.L			
			Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	59940	020307-83-9	91
			Cyclohexene, 3-(1,5-dimethyl-4-hex	59945	020307-83-9	83

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			enyl)-6-methylene-, [S-(R*,S*)]-			
			1S-.alpha.-Pinene	15187	007785-26-4	35
8	22.225	2.56	C:\Database\NIST05a.L			
			1H-3a,7-Methanoazulene, 2,3,4,7,8,	60060	000469-61-4	91
			8a-hexahydro-3,6,8,8-tetramethyl-,			
			[3R-(3.alpha.,3a.beta.,7.beta.,8a			
			.alpha.)]-			
			Tricyclo[5.4.0.0(2,8)]undec-9-ene,	59908	005989-08-2	90
			2,6,6,9-tetramethyl-			
			1H-3a,7-Methanoazulene, 2,3,4,7,8,	60059	000469-61-4	87
			8a-hexahydro-3,6,8,8-tetramethyl-,			
			[3R-(3.alpha.,3a.beta.,7.beta.,8a			
			.alpha.)]-			
9	22.709	4.24	C:\Database\NIST05a.L			
			4,7-Methanoazulene, 1,2,3,4,5,6,7,	60012	000514-51-2	64
			8-octahydro-1,4,9,9-tetramethyl-,			
			[1S-(1.alpha.,4.alpha.,7.alpha.)]-			
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct	60068	030021-74-0	60
			ahydro-7-methyl-4-methylene-1-(1-m			
			ethylethyl)-, (1.alpha.,4a.alpha.,			
			8a.alpha.)-			
			1H-3a,7-Methanoazulene, 2,3,4,7,8,	60059	000469-61-4	56
			8a-hexahydro-3,6,8,8-tetramethyl-,			
			[3R-(3.alpha.,3a.beta.,7.beta.,8a			
			.alpha.)]-			
10	23.152	2.82	C:\Database\NIST05a.L			
			Benzeneethanamine, .alpha.,2,6-tri	31454	057204-69-0	35
			methyl-, (.+/-.)-			
			2,3-Dimethylamphetamine	31416	075659-60-8	27
			3-Cyclohexene-1-methanol, .alpha.,	72995	023178-88-3	16
			4-dimethyl-.alpha.-(4-methyl-3-pen			
			tenyl)-, [R-(R*,R*)]-			
11	24.035	2.81	C:\Database\NIST05a.L			
			1H-3a,7-Methanoazulene, 2,3,4,7,8,	60060	000469-61-4	83
			8a-hexahydro-3,6,8,8-tetramethyl-,			
			[3R-(3.alpha.,3a.beta.,7.beta.,8a			
			.alpha.)]-			
			Naphthalene, 1,2,3,4,4a,7-hexahydr	59947	016728-99-7	70
			o-1,6-dimethyl-4-(1-methylethyl)-			
			1H-Benzocycloheptene, 2,4a,5,6,7,8	59959	001461-03-6	62
			-hexahydro-3,5,5,9-tetramethyl-, (			

Figure B.1: Spectrum and Compounds of Ginger (*Zingiber Officinale* Roscoe) essential oil under 30 minutes extraction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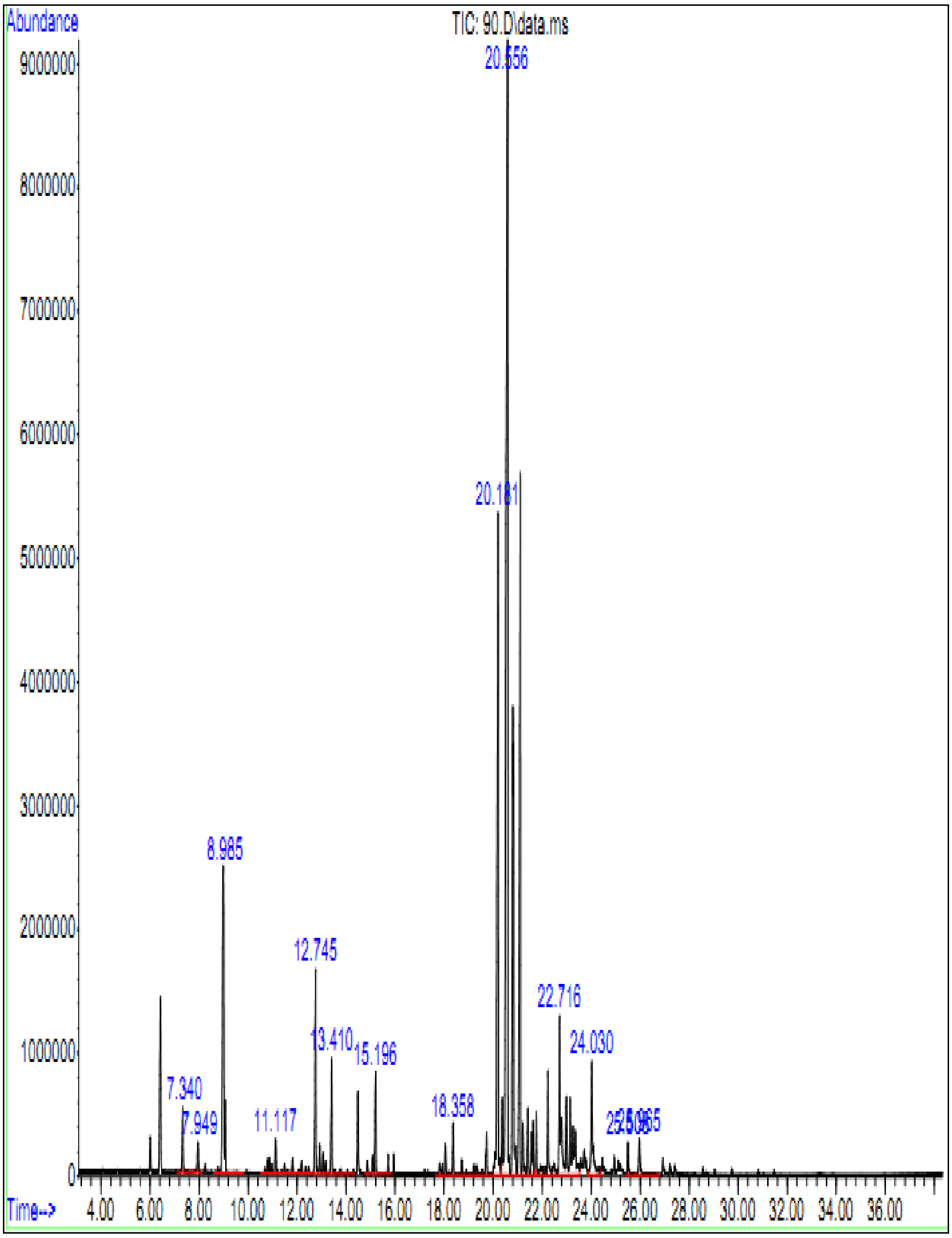
13.409
22.713
24.032
23.157
7.946
11.116
18.358
25.966
6500000
6000000
5500000
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
Time-->
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	6.416	4.41	C:\Database\NIST05a.L			
			Camphene	15152	000079-92-5	97
			Camphene	15161	000079-92-5	96
			Camphene	15160	000079-92-5	95
2	7.946	0.76	C:\Database\NIST05a.L			
			.beta.-Pinene	15176	000127-91-3	76
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	15374	028634-89-1	76
			Betahistine	15649	005638-76-6	59
3	8.985	8.99	C:\Database\NIST05a.L			
			.beta.-Phellandrene	15198	000555-10-2	46
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	15374	028634-89-1	46
			.beta.-Phellandrene	15200	000555-10-2	46
4	11.117	1.83	C:\Database\NIST05a.L			
			Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-, (1S)-	15369	000498-15-7	42
			1S-.alpha.-Pinene	15185	007785-26-4	42
			Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	15345	000488-97-1	42
5	12.746	5.06	C:\Database\NIST05a.L			
			Borneol	25495	000507-70-0	94
			Borneol	25498	000507-70-0	91
			Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	25824	000464-45-9	90
6	13.408	2.27	C:\Database\NIST05a.L			
			3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl-	25797	000098-55-5	90
			3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl-	25798	000098-55-5	90
			3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl-	25796	000098-55-5	90
7	15.196	3.11	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	96
			2,6-Octadienal, 3,7-dimethyl-	24108	005392-40-5	95
			2,6-Octadienal, 3,7-dimethyl-, (E)	24151	000141-27-5	95
8	18.360	1.83	C:\Database\NIST05a.L			
			Cyclohexane, 1-ethenyl-1-methyl-2,	59911	110823-68-2	91

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			4-bis(1-methylethenyl)-			
			Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, [1S-(1.al pha.,2.beta.,4.beta.)]-	60003	000515-13-9	91
			Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, [1S-(1.al pha.,2.beta.,4.beta.)]-	60001	000515-13-9	90
9	20.532	34.74	C:\Database\NIST05a.L			
			1,3-Cyclohexadiene, 5-(1,5-dimethy l-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	59958	000495-60-3	91
			1,3-Cyclohexadiene, 5-(1,5-dimethy l-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	59955	000495-60-3	90
			Bicyclo[3.1.1]hept-2-ene, 2,6-dime thyl-6-(4-methyl-3-pentenyl)-	59930	017699-05-7	87
10	20.795	8.59	C:\Database\NIST05a.L			
			1,6,10-Dodecatriene, 7,11-dimethyl -3-methylene-, (E)-	59898	018794-84-8	94
			Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	59929	000495-61-4	70
			Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	59931	000495-61-4	60
11	21.068	12.26	C:\Database\NIST05a.L			
			Cyclohexene, 3-(1,5-dimethyl-4-hex enyl)-6-methylene-, [S-(R*,S*)]-	59940	020307-83-9	93
			Cyclohexene, 3-(1,5-dimethyl-4-hex enyl)-6-methylene-, [S-(R*,S*)]-	59945	020307-83-9	86
			2-Butenamide,N-phenyl-	30128	001733-40-0	30
12	22.713	7.78	C:\Database\NIST05a.L			
			1,6,10-Dodecatriene, 7,11-dimethyl -3-methylene-, (E)-	59898	018794-84-8	83
			1H-3a,7-Methanoazulene, 2,3,4,7,8, 8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a .alpha.)]-	60059	000469-61-4	66
			1H-3a,7-Methanoazulene, 2,3,4,7,8, 8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a .alpha.)]-	60060	000469-61-4	66

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
13	23.156	3.16	C:\Database\NIST05a.L			
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58537	000644-30-4	40
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58536	000644-30-4	38
			1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	59895	028973-97-9	35
14	24.032	3.44	C:\Database\NIST05a.L			
			1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	60060	000469-61-4	93
			Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	59947	016728-99-7	78
			1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	60059	000469-61-4	76
15	25.968	1.78	C:\Database\NIST05a.L			
			1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane	71449	1000144-09-7	38
			1-Formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclohexene	71425	1000144-10-1	27
			1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	72989	1000144-10-6	27

Figure B.2: Spectrum and Compounds of Ginger (*Zingiber Officinale* Roscoe) essential oil under 60 minutes extraction



Abundance
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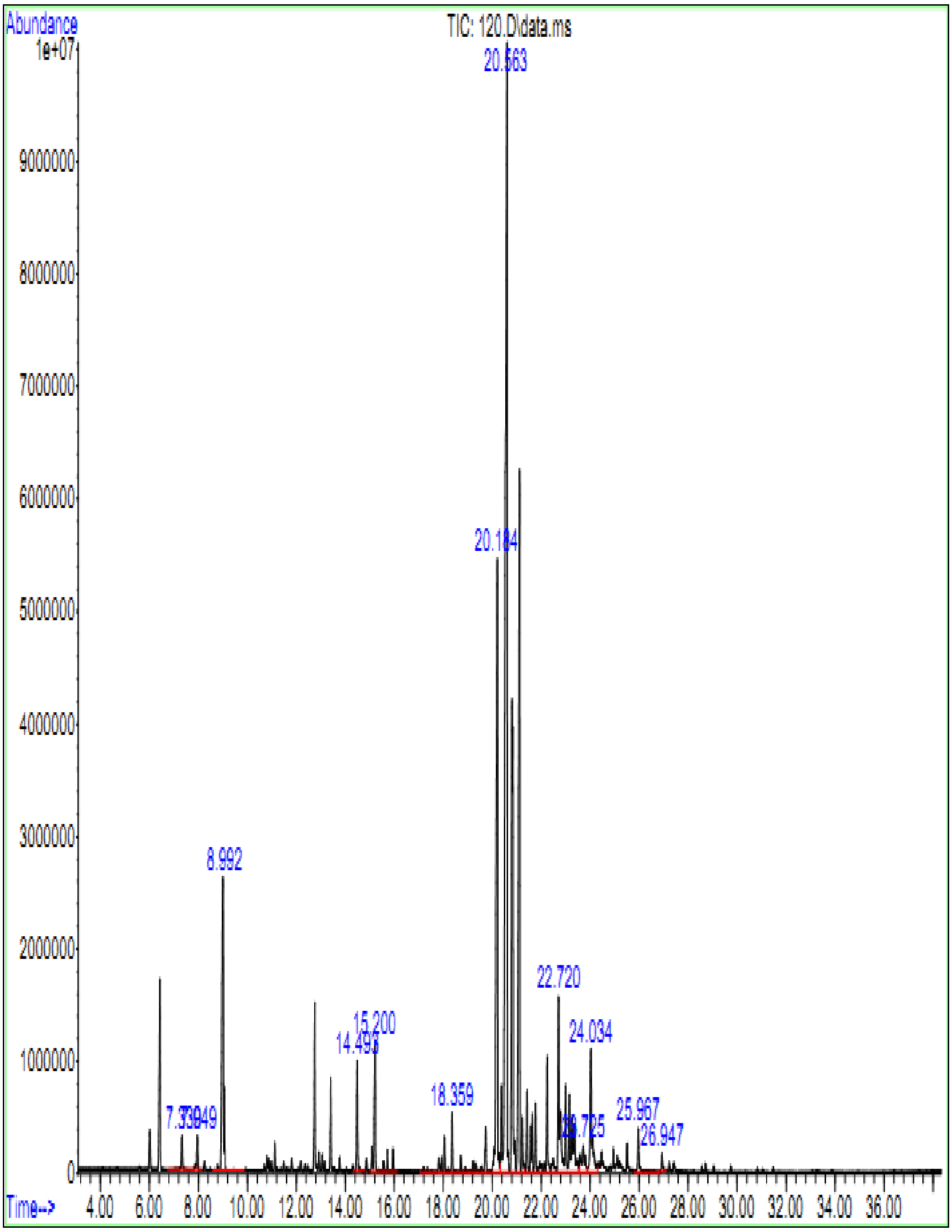
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
Time-->
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00
20.556
20.181
8.985
12.745
22.716
24.030
13.410
15.196
7.340
7.949
11.117
18.358

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	7.340	1.05	C:\Database\NIST05a.L			
			5-Hepten-2-one, 6-methyl-	11018	000110-93-0	50
			5-Hepten-2-one, 6-methyl-	11033	000110-93-0	49
			5-Hepten-2-one, 6-methyl-	11034	000110-93-0	35
2	7.950	0.46	C:\Database\NIST05a.L			
			.beta.-Myrcene	15179	000123-35-3	55
			Betahistine	15649	005638-76-6	53
			Bicyclo[3.1.0]hex-2-ene, 4-methyl- 1-(1-methylethyl)-	15374	028634-89-1	53
3	8.985	6.92	C:\Database\NIST05a.L			
			.beta.-Phellandrene	15198	000555-10-2	46
			Bicyclo[3.1.0]hex-2-ene, 4-methyl- 1-(1-methylethyl)-	15374	028634-89-1	46
			.beta.-Phellandrene	15200	000555-10-2	46
4	11.117	1.37	C:\Database\NIST05a.L			
			3-Carene	15151	013466-78-9	83
			Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-, (1S)-	15369	000498-15-7	60
			1,3,7-Octatriene, 3,7-dimethyl-	15243	000502-99-8	58
5	12.746	4.17	C:\Database\NIST05a.L			
			Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	25824	000464-45-9	90
			Borneol	25499	010385-78-1	90
			Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	25830	000464-45-9	90
6	13.412	1.68	C:\Database\NIST05a.L			
			3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl-	25796	000098-55-5	90
			3-Methyl-trans-3a,4,7,7a-tetrahydroindane	15309	1000145-84-3	86
			3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl-	25798	000098-55-5	80
7	15.196	1.93	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	96
			2,6-Octadienal, 3,7-dimethyl-, (E)	24151	000141-27-5	94
			2,6-Octadienal, 3,7-dimethyl-, (E)	24141	000141-27-5	94
8	18.360	1.59	C:\Database\NIST05a.L			
			Cyclohexane, 1-ethenyl-1-methyl-2,	60003	000515-13-9	91

```
Pk#    RT  Area%        Library/ID              Ref#    CAS#   Qual
______________________________________________________________________
             4-bis(1-methylethenyl)-, [1S-(1.al
             pha.,2.beta.,4.beta.)]-
             Cyclohexane, 1-ethenyl-1-methyl-2,  60001 000515-13-9 90
             4-bis(1-methylethenyl)-, [1S-(1.al
             pha.,2.beta.,4.beta.)]-
             1,Z-5,E-7-Dodecatriene           32175 083085-83-0 74

 9  20.181 13.68 C:\Database\NIST05a.L
             Benzene, 1-(1,5-dimethyl-4-hexenyl  58537 000644-30-4 98
             )-4-methyl-
             Benzene, 1-(1,5-dimethyl-4-hexenyl  58538 000644-30-4 95
             )-4-methyl-
             Benzene, 1-(1,5-dimethyl-4-hexenyl  58536 000644-30-4 93
             )-4-methyl-

10  20.555 51.26 C:\Database\NIST05a.L
             1,3-Cyclohexadiene, 5-(1,5-dimethy  59958 000495-60-3 94
             l-4-hexenyl)-2-methyl-, [S-(R*,S*)
             ]-
             1,3-Cyclohexadiene, 5-(1,5-dimethy  59955 000495-60-3 90
             l-4-hexenyl)-2-methyl-, [S-(R*,S*)
             ]-
             trans-(2-Chlorovinyl)trimethylsila  14568 1000139-55-2 59
             ne

11  22.716 11.40 C:\Database\NIST05a.L
             1H-3a,7-Methanoazulene, 2,3,4,7,8,  60059 000469-61-4 66
             8a-hexahydro-3,6,8,8-tetramethyl-,
              [3R-(3.alpha.,3a.beta.,7.beta.,8a
             .alpha.)]-
             Di-epi-.alpha.-cedrene            59852 1000156-13-3 60
             1H-3a,7-Methanoazulene, 2,3,4,7,8,  60060 000469-61-4 59
             8a-hexahydro-3,6,8,8-tetramethyl-,
              [3R-(3.alpha.,3a.beta.,7.beta.,8a
             .alpha.)]-

12  24.032  3.04 C:\Database\NIST05a.L
             1H-3a,7-Methanoazulene, 2,3,4,7,8,  60059 000469-61-4 86
             8a-hexahydro-3,6,8,8-tetramethyl-,
              [3R-(3.alpha.,3a.beta.,7.beta.,8a
             .alpha.)]-
             1H-3a,7-Methanoazulene, 2,3,4,7,8,  60061 000469-61-4 81
             8a-hexahydro-3,6,8,8-tetramethyl-,
              [3R-(3.alpha.,3a.beta.,7.beta.,8a
             .alpha.)]-
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	60056	000469-61-4	74
13	25.506	0.60	C:\Database\NIST05a.L			
			Sesquirosefuran	69927	039007-93-7	43
			6-Methyl-6,7-dihydro-9H-5-oxa-9-azabenzocyclohepten-8-one	40911	1000210-20-2	37
			3,7,11-Trimethyl-dodeca-2,4,6,10-tetraenal	69946	013832-89-8	35
14	25.965	0.84	C:\Database\NIST05a.L			
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58536	000644-30-4	59
			1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane	71449	1000144-09-7	30
			1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	72989	1000144-10-6	30

Figure B.3: Spectrum and Compounds of Ginger (*Zingiber Officinale* Roscoe) essential oil under 90 minutes extraction



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6000000
5000000
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3000000
2000000
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0
Time-->
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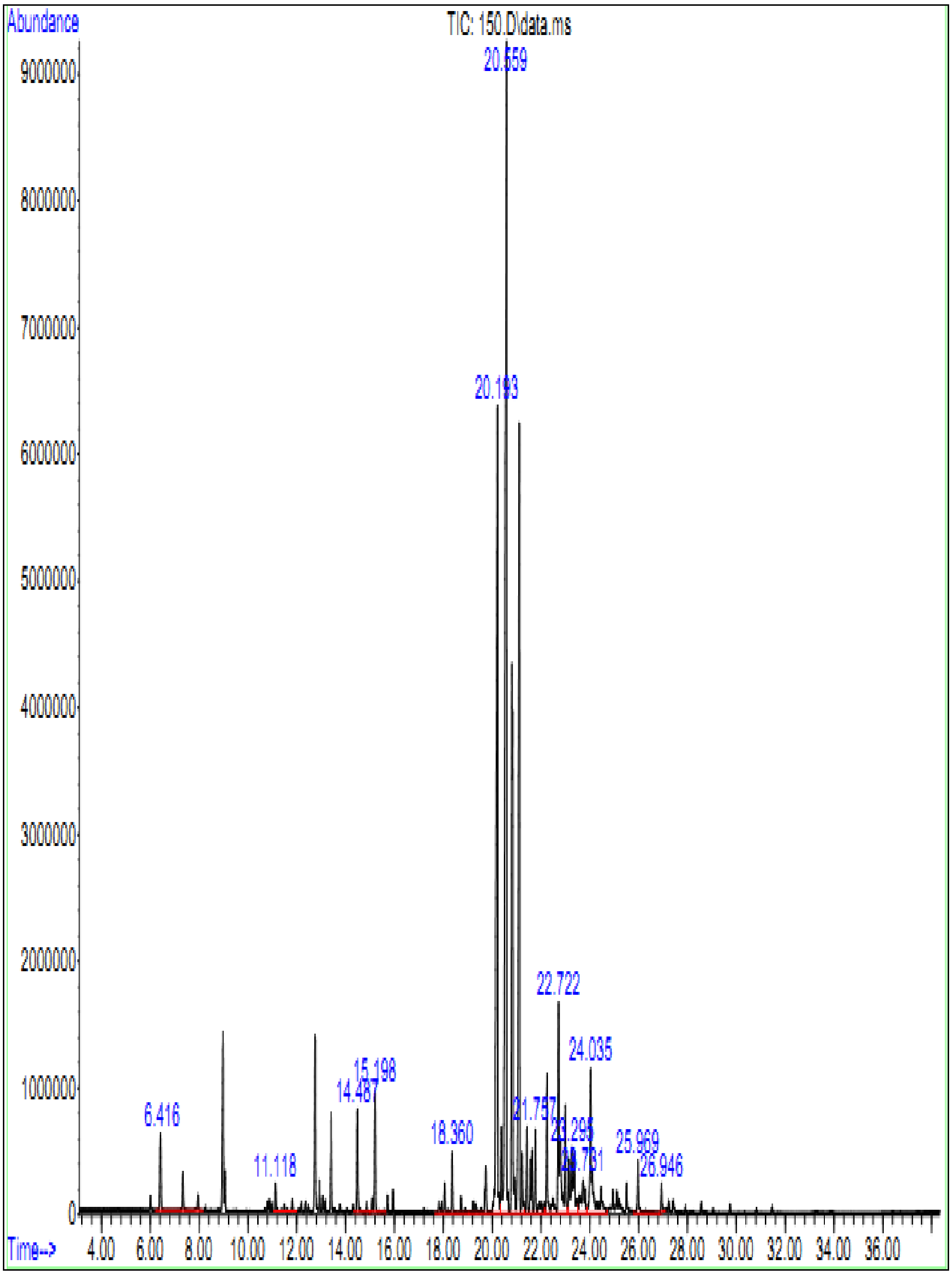
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00
20.563
20.184
8.992
22.720
15.200
14.493
24.034
18.359
25.967
26.947

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	7.329	0.58	C:\Database\NIST05a.L			
			.beta.-Myrcene	15179	000123-35-3	59
			5-Hepten-2-one, 6-methyl-	11034	000110-93-0	41
			5-Hepten-2-one, 6-methyl-	11018	000110-93-0	38
2	7.950	0.54	C:\Database\NIST05a.L			
			Bicyclo[3.1.0]hex-2-ene, 4-methyl- 1-(1-methylethyl)-	15374	028634-89-1	64
			Betahistine	15649	005638-76-6	64
			.beta.-Phellandrene	15198	000555-10-2	62
3	8.992	6.91	C:\Database\NIST05a.L			
			Bicyclo[3.1.0]hex-2-ene, 4-methyl- 1-(1-methylethyl)-	15374	028634-89-1	60
			.beta.-Phellandrene	15200	000555-10-2	60
			Cyclohexene, 4-methylene-1-(1-meth ylethyl)-	15324	000099-84-3	60
4	14.494	1.46	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-, (Z)	24148	000106-26-3	64
			2,6-Octadienal, 3,7-dimethyl-, (Z)	24150	000106-26-3	50
			1,6-Octadiene, 3,7-dimethyl-, (S)-	16373	010281-55-7	35
5	15.200	2.85	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	97
			2,6-Octadienal, 3,7-dimethyl-	24102	005392-40-5	95
			2,6-Octadienal, 3,7-dimethyl-, (E)	24141	000141-27-5	94
6	18.360	1.97	C:\Database\NIST05a.L			
			Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, [1S-(1.al pha.,2.beta.,4.beta.)]-	60003	000515-13-9	90
			1,Z-5,E-7-Dodecatriene	32175	083085-83-0	74
			8-Isopropenyl-1,5-dimethyl-cyclode ca-1,5-diene	59885	1000193-62-7	68
7	20.185	13.50	C:\Database\NIST05a.L			
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58537	000644-30-4	98
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58538	000644-30-4	95
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58536	000644-30-4	93
8	20.562	51.59	C:\Database\NIST05a.L			

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	59958	000495-60-3	91
			1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	59955	000495-60-3	90
			Benzene, 1-(1,5-dimethylhexyl)-4-methyl-	59877	001461-02-5	50
9	22.720	14.65	C:\Database\NIST05a.L			
			Di-epi-.alpha.-cedrene	59852	1000156-13-3	86
			1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	60060	000469-61-4	66
			1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	60059	000469-61-4	64
10	23.725	1.25	C:\Database\NIST05a.L			
			Aromadendrene, dehydro-	58517	1000156-12-5	60
			Isolongifolene, 9,10-dehydro-	58523	1000151-67-1	52
			Neoisolongifolene, 8,9-dehydro-	58527	067517-14-0	46
11	24.036	3.47	C:\Database\NIST05a.L			
			1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	60059	000469-61-4	91
			1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	60061	000469-61-4	87
			1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	60060	000469-61-4	87
12	25.968	0.95	C:\Database\NIST05a.L			
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58536	000644-30-4	53
			1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane	71449	1000144-09-7	38

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol	113279	1000144-12-4	27
13	26.947	0.28	C:\Database\NIST05a.L			
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58537	000644-30-4	95
			Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-, (R)-	58542	016982-00-6	70
			Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-, (R)-	58541	016982-00-6	70

Figure B.4: Spectrum and Compounds of Ginger (*Zingiber Officinale* Roscoe) essential oil under 120minutes extraction



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21.757
23.295
25.969
26.946
9000000
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
Time-->
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
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30.00
32.00
34.00
36.00

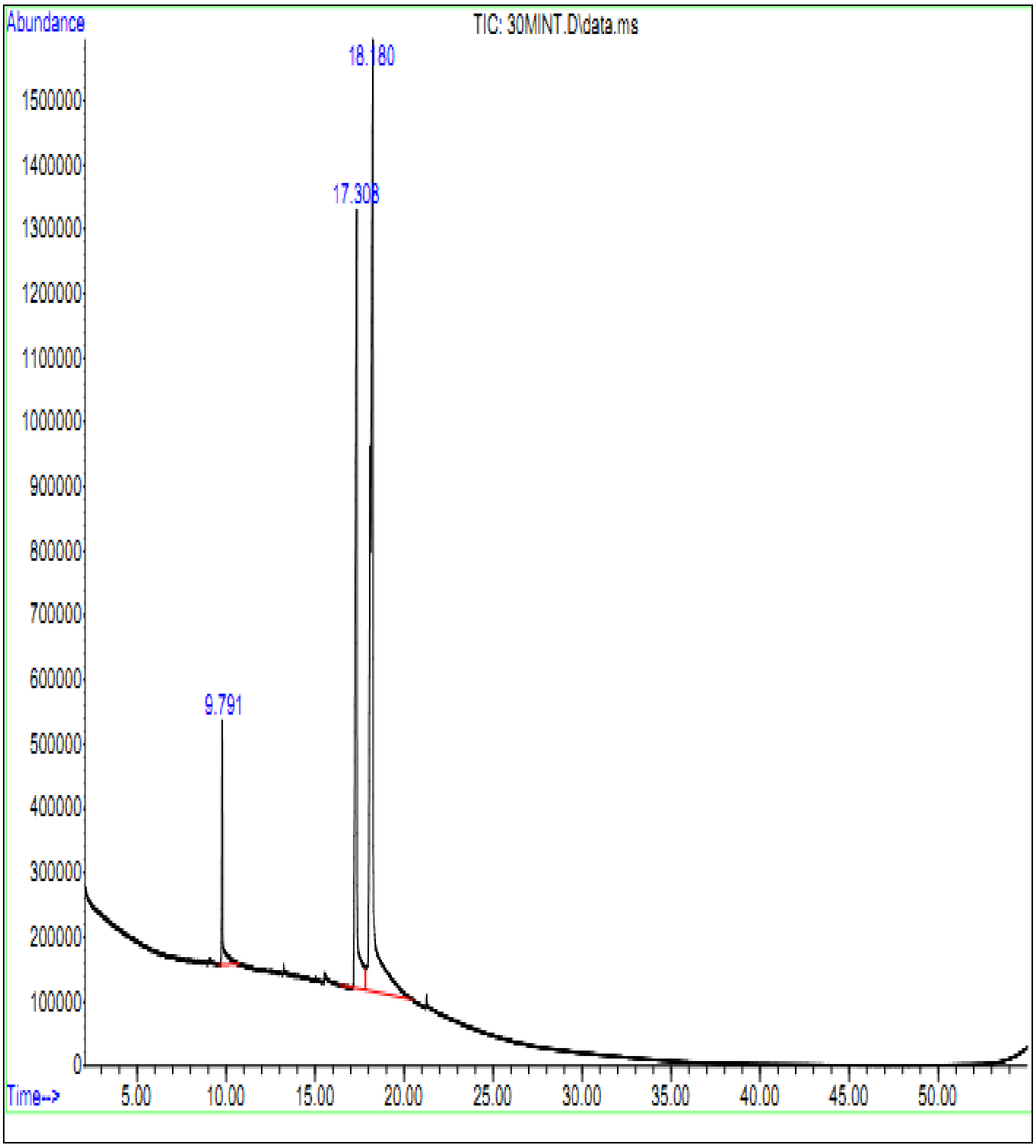
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	6.416	1.90	C:\Database\NIST05a.L			
			Camphene	15152	000079-92-5	97
			Camphene	15160	000079-92-5	96
			Camphene	15161	000079-92-5	96
2	11.117	0.57	C:\Database\NIST05a.L			
			Bicyclo[4.1.0]hept-3-ene, 3,7,7-tr imethyl-, (1S)-	15369	000498-15-7	93
			3-Carene	15156	013466-78-9	92
			3-Carene	15151	013466-78-9	83
3	14.487	1.40	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-, (Z)	24148	000106-26-3	60
			1,3,8-p-Menthatriene	14349	021195-59-5	38
			2-Cyclohexen-1-ol, 2-methyl-5-(1-m ethylethenyl)-	24259	000099-48-9	35
4	15.200	1.77	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	95
			2,6-Octadienal, 3,7-dimethyl-, (E)	24151	000141-27-5	94
			2,6-Octadienal, 3,7-dimethyl-	24102	005392-40-5	94
5	18.360	1.61	C:\Database\NIST05a.L			
			Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, [1S-(1.al pha.,2.beta.,4.beta.)]-	60003	000515-13-9	91
			Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-	59911	110823-68-2	90
			Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, [1S-(1.al pha.,2.beta.,4.beta.)]-	60001	000515-13-9	90
6	20.192	16.61	C:\Database\NIST05a.L			
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58537	000644-30-4	98
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58538	000644-30-4	95
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58536	000644-30-4	95
7	20.558	52.09	C:\Database\NIST05a.L			
			1,3-Cyclohexadiene, 5-(1,5-dimethy l-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	59958	000495-60-3	91
			1,3-Cyclohexadiene, 5-(1,5-dimethy	59955	000495-60-3	90

```
Pk#    RT  Area%          Library/ID              Ref#     CAS#   Qual
------------------------------------------------------------------------
              l-4-hexenyl)-2-methyl-, [S-(R*,S*)
              ]-
              Benzene, 1-(1,5-dimethylhexyl)-4-m  59877 001461-02-5 45
              ethyl-

 8  21.756  4.14 C:\Database\NIST05a.L
              .alpha.-Farnesene                59834 000502-61-4 93
              Cyclohexene, 1-methyl-4-(5-methyl-  59932 000495-61-4 83
              1-methylene-4-hexenyl)-, (S)-
              1,3,6,10-Dodecatetraene, 3,7,11-tr  59890 026560-14-5 80
              imethyl-, (Z,E)-

 9  22.724  8.69 C:\Database\NIST05a.L
              Di-epi-.alpha.-cedrene            59852 1000156-13-3 70
              1H-3a,7-Methanoazulene, 2,3,4,7,8,  60060 000469-61-4 66
              8a-hexahydro-3,6,8,8-tetramethyl-,
               [3R-(3.alpha.,3a.beta.,7.beta.,8a
              .alpha.)]-
              1H-3a,7-Methanoazulene, 2,3,4,7,8,  60059 000469-61-4 64
              8a-hexahydro-3,6,8,8-tetramethyl-,
               [3R-(3.alpha.,3a.beta.,7.beta.,8a
              .alpha.)]-

10  23.297  3.34 C:\Database\NIST05a.L
              1H-Cyclopropa[a]naphthalene, decah  60072 020071-49-2 97
              ydro-1,1,3a-trimethyl-7-methylene-
              , [1aS-(1a.alpha.,3a.alpha.,7a.bet
              a.,7b.alpha.)]-
              Naphthalene, 1,2,3,5,6,7,8,8a-octa  60051 010219-75-7 95
              hydro-1,8a-dimethyl-7-(1-methyleth
              enyl)-, [1S-(1.alpha.,7.alpha.,8a.
              alpha.)]-
              .beta.-Guaiene                   59807 000088-84-6 95

11  23.733  1.54 C:\Database\NIST05a.L
              Isolongifolene, 9,10-dehydro-      58523 1000151-67-1 91
              .beta.-Vatirenene                 58513 1000293-04-2 89
              Aromadendrene, dehydro-            58517 1000156-12-5 50

12  24.036  4.84 C:\Database\NIST05a.L
              1H-3a,7-Methanoazulene, 2,3,4,7,8,  60060 000469-61-4 87
              8a-hexahydro-3,6,8,8-tetramethyl-,
               [3R-(3.alpha.,3a.beta.,7.beta.,8a
              .alpha.)]-
              1H-3a,7-Methanoazulene, 2,3,4,7,8,  60059 000469-61-4 81
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a .alpha.)]-			
			Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-	59908	005989-08-2	64
13	25.968	1.07	C:\Database\NIST05a.L			
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58536	000644-30-4	64
			1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane	71449	1000144-09-7	30
			1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	59890	026560-14-5	15
14	26.948	0.42	C:\Database\NIST05a.L			
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58537	000644-30-4	90
			Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	58536	000644-30-4	90
			Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-, (R)-	58540	016982-00-6	38

Figure B.5: Spectrum and Compounds of Ginger (*Zingiber Officinale* Roscoe) essential oil under 150 minutes extraction

Appendix B.2: Effect of Extraction Time on Chemical Constituents of Essential Oil of Lemongrass *(Cymbopogon citratus)*



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
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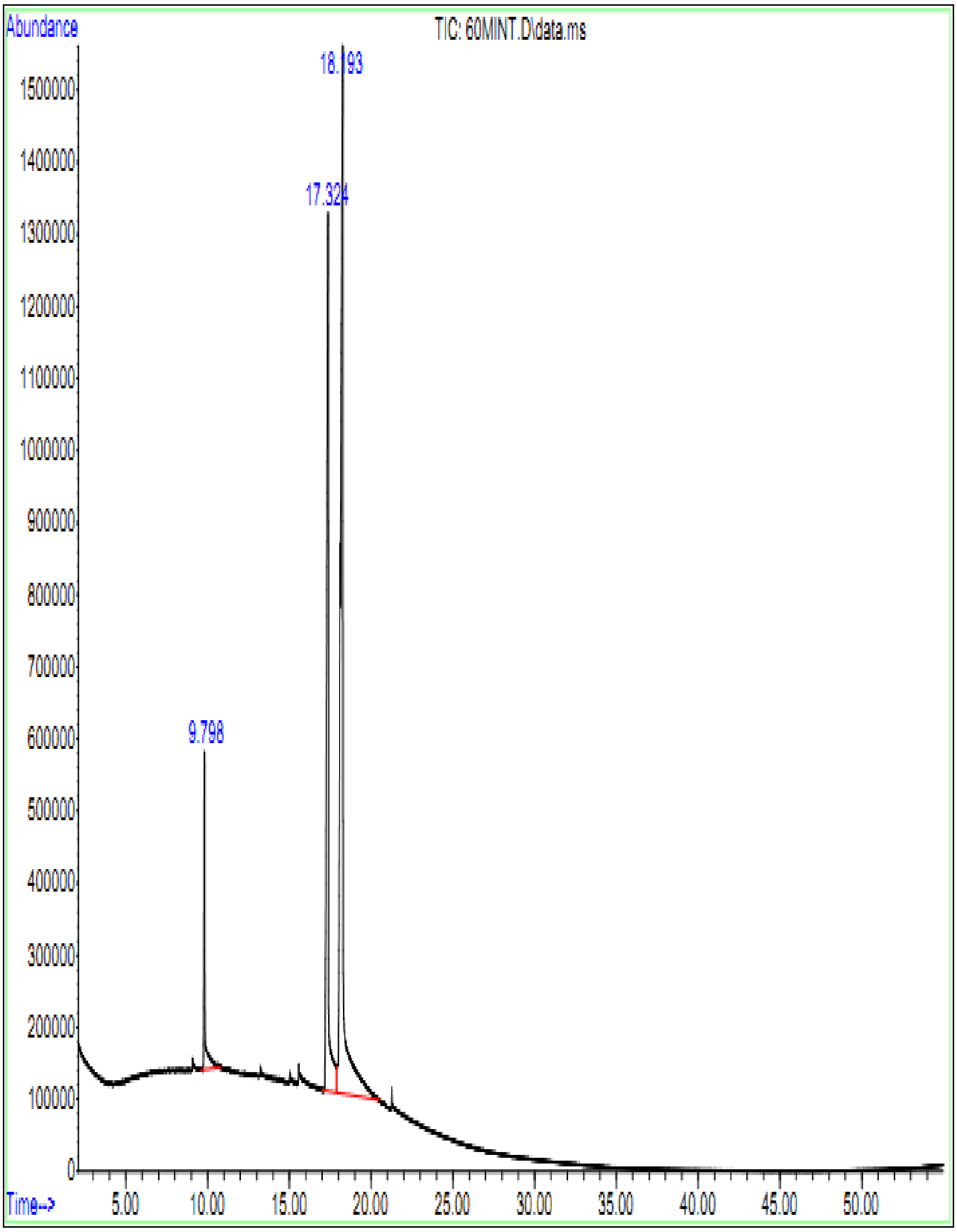
			Ethanamine, 2-(methylthio)-	2339	018542-42-2	3
			1-Propanamine, 3-chloro-	2427	014753-26-5	3
			2-Methyl-1,5-(4H)-dihydropyrido-(2 ,3-b)1,4-diazepine-4-one	39983	017260-05-8	2
2	17.309	32.79	C:\Database\NIST05a.L			
			2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	14438	000460-01-5	55
			Tetracyclo[3.3.1.1(1,8).0(2,4)]dec ane	14433	1000185-58-7	46
			2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	14437	000460-01-5	45
3	18.181	62.00	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24102	005392-40-5	96
			2,6-Octadienal, 3,7-dimethyl-, (E)	24151	000141-27-5	96
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	95

Figure B.6: Spectrum and Compounds of Lemongrass *(Cymbopogon citratus)* essential oil under 30 minutes extraction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700000
600000
500000
400000
300000
200000
100000
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Time-->
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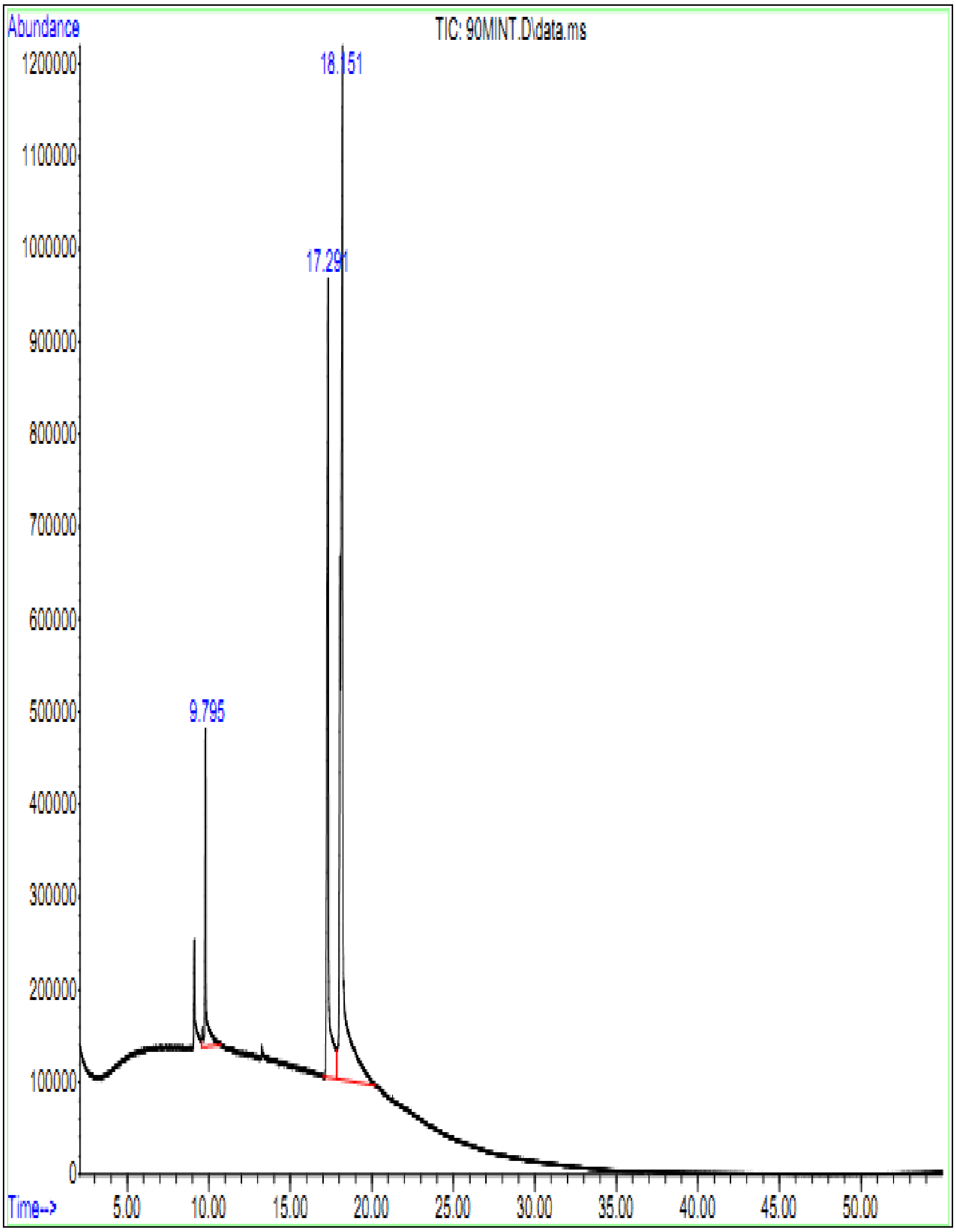
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00

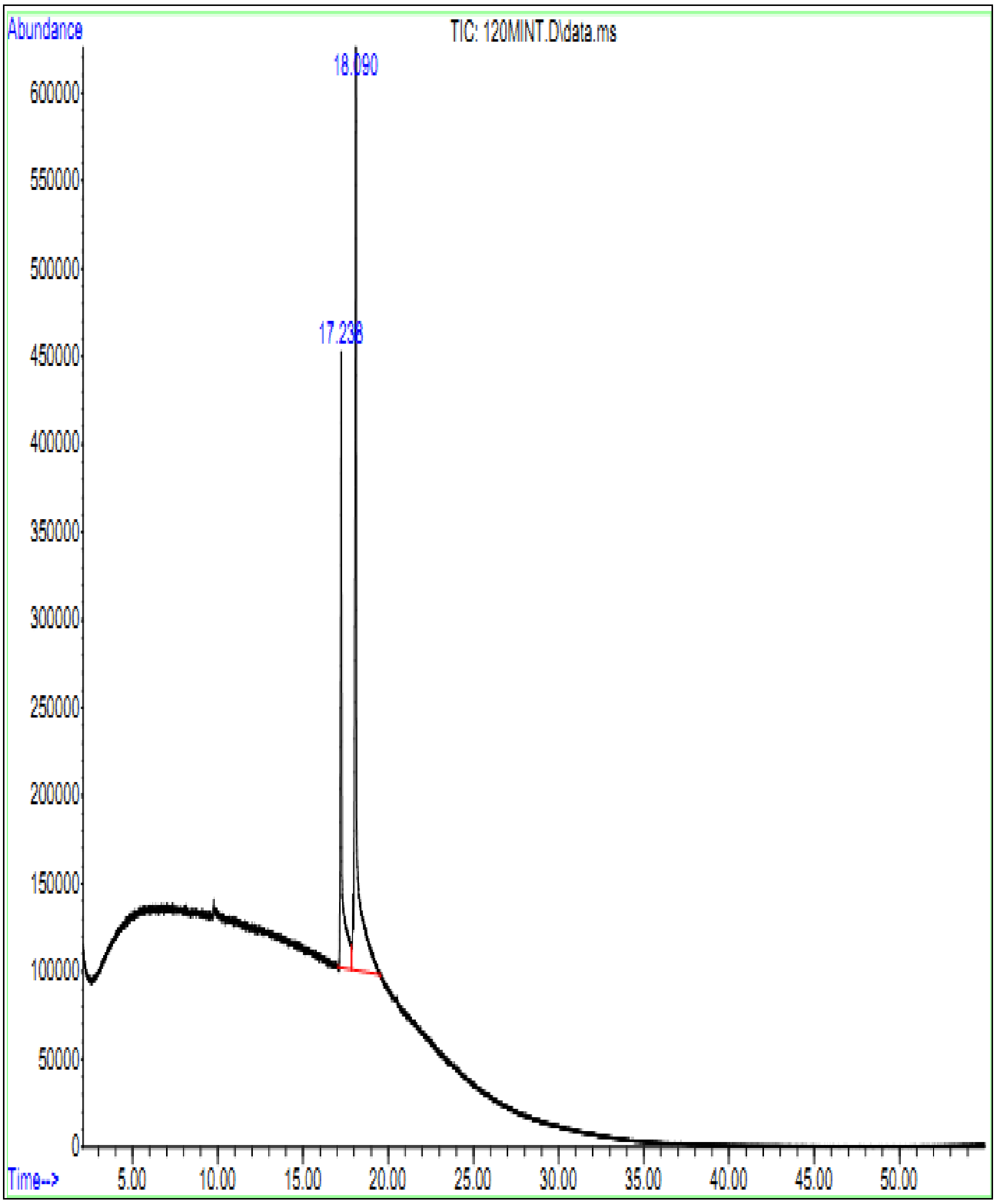
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	9.800	6.16	C:\Database\NIST05a.L			
			Phenylethanolamine	16140	007568-93-6	9
			dl-2-Amino-1-phenylethanol	16178	001936-63-6	4
			1-Propanamine, 3-chloro-	2427	014753-26-5	3
2	17.325	33.84	C:\Database\NIST05a.L			
			Cycloheptane, 1,3,5-tris(methylene)-	14418	068284-24-2	58
			2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	14438	000460-01-5	55
			2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	14437	000460-01-5	45
3	18.192	60.01	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24102	005392-40-5	96
			2,6-Octadienal, 3,7-dimethyl-, (E)	24151	000141-27-5	95
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	94

Figure B.7: Spectrum and Compounds of Lemongrass *(Cymbopogon citratus)* essential oil under 60 minutes extraction


Abundance
TIC: 90MINT.D\data.ms
18.151
17.291
9.795
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
Time-->
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	9.794	7.94	C:\Database\NIST05a.L			
			Ethanamine, 2-(methylthio)-	2339	018542-42-2	3
			2-Methyl-1,5-(4H)-dihydropyrido-(2,3-b)1,4-diazepine-4-one	39983	017260-05-8	2
			Benzeneethanamine	9265	000064-04-0	2
2	17.293	30.63	C:\Database\NIST05a.L			
			Acetonitrile, bromo-	8721	000590-17-0	3
			Glycine, N-acetyl-	8209	000543-24-8	3
			Acetic acid, trichloro-, ethyl ester	50072	000515-84-4	2
3	18.149	61.44	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24102	005392-40-5	96
			2,6-Octadienal, 3,7-dimethyl-, (E)	24151	000141-27-5	95
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	94

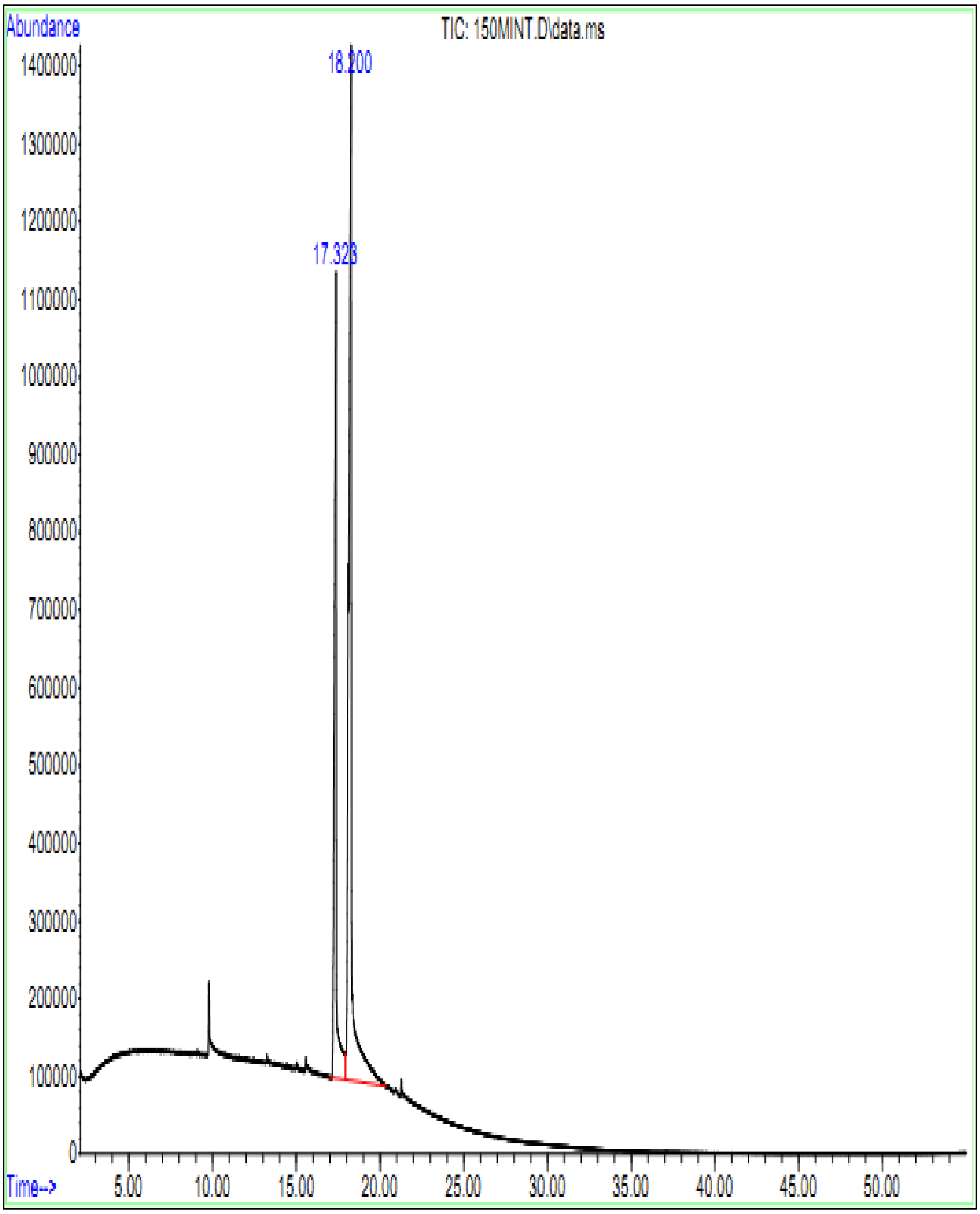
Figure B.8: Spectrum and Compounds of Lemongrass *(Cymbopogon citratus)* essential oil under 90 minutes extraction



Abundance
TIC: 120MINT.D\data.ms
18.090
17.238
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
Time-->
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	17.239	32.18	C:\Database\NIST05a.L			
			Phenol, 2,6-dinitro-4-(trifluoromethyl)-	93625	000393-77-1	7
			Benzeneethanamine	9265	000064-04-0	7
			Acetonitrile, bromo-	8721	000590-17-0	5
2	18.090	67.82	C:\Database\NIST05a.L			
			Phenol, 2,6-dinitro-4-(trifluoromethyl)-	93625	000393-77-1	9
			3,5-Dimethoxyphenethylamine	43745	003213-28-3	9
			2H-Pyran, 2-(3-butynyloxy)tetrahydro-	26517	040365-61-5	9

Figure B.9: Spectrum and Compounds of Lemongrass *(Cymbopogon citratus)* essential oil under 120 minutes extraction



Abundance
TIC: 150MINT.D\data.ms
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17.328
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
Time-->
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	17.325	34.20	C:\Database\NIST05a.L			
			2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	14438	000460-01-5	55
			Cycloheptane, 1,3,5-tris(methylene)-	14418	068284-24-2	49
			6-Isopropenyl-3-methoxymethoxy-3-methyl-cyclohexene	54298	1000188-35-8	42
2	18.202	65.80	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24102	005392-40-5	96
			2,6-Octadienal, 3,7-dimethyl-, (E)	24151	000141-27-5	96
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	95

Figure B.10: Spectrum and Compounds of Lemongrass *(Cymbopogon citratus)* essential oil under 150 minutes extraction