ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTES OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

OPTIMAZATION AND CHARACTERIZATION OF EXTRACT ESSENTIAL OIL FROM ZINGIBER OFFICINALE AND EVALUATE ANTIMICROBIAL EFFECT

Thesis Submitted to Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemical Engineering (Process Engineering Stream)

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PROCESS ENGINEERING STREAM

This is to certify that the thesis prepared by Fire Chewaka, entitled: Production of crude ginger oil from *Zingiber Officinale* Roscoe(ginger) root and submitted in partial fulfillment of the requirements for the Degree of Master of Science in process engineering, complies with the regulations of the university and meets the accepted standards with respect to the originality and quality.

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List of Acronyms

AAs: Atomic Absorption Spectrophotometers

ANOVA: Analysis of Variance

AOAC: Association of Official Analytical Chemistry

BC: Before Christ

CCD: Central Composite Design

DNA: Deoxyribonucleic Acid

EFMHACA: Ethiopia Food, Medicine and Health care Administration and Control Authority

GC – MS: Gas Chromatography Mass Spectrophotometer

GDP: Gross Domestic Product

GPx: Glutathione peroxide

MAE: Microwave Assisted Extraction

NASA: The National Aeronautics and Space Administration

OFAT: One Factor at Time

PFE: Pressurized Fluid Extraction

PMAE: pressurized microwave-assisted extraction

PLE: Pressurized Liquid Extraction

RNS: Reactive Nitrogen species

ROS: Reactive Oxygen species

RSM: Response Surface Methodology

FC: Supercritical fluid chromatography

SFE: Supercritical fluid extraction

SFMAE: Solvent-free microwave-assisted extraction

SOD: Superoxide dismutase

TCM: Traditional Chinese medicine

UV: Ultra Violet

ABSTRACT

The demand projection in Ethiopia divulges that the domestic demands for essential oils are substantially increasing with time. This increasing demand of essential oil, such as ginger oil has opened up wide opportunities for global and local market and this leads to identify an optimum extraction technique to produce higher quality of a crude oil with higher yield locally. The parameters selected for the study was different solvent ratio with amount of 160ml, 200ml and 240ml, the particle size of 1 µm, 3µm and 5µm and also with residence time of 1hr, 2hr and 3hr. Response Surface Methodology (RSM) with Central Composite Design (CCD) with three levels factorial design is the statistical analysis used for optimization process of ginger oil yield. From the result obtained the final optimization value for high yield of ginger oil is at solvent ratio of 160 ml to 20 grams sample at 3hr residence time and the particle size is 3 µm and the major compound in ginger oil is zingiberene with 29.74% value and also the effect of ginger oil on antimicrobial shows the positive result. In conclusion, this study can be in food and medicinal sectors whose interested to engage in manufacturing natural antioxidant from ginger oil and also the results indicate it can substitutes the import synthetic preservative and save hard currency for the country.

CHAPTER ONE

1. Introduction

1.1 Background

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 [1]. Essential oils are found in diverse plant parts 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 also 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 [2]. `

Essential oils are one of the demanded natural products in the local or international market and are extracted from many different plant parts. Essential oils as a group do not have any specific chemical or pharmaceutical properties in common. Instead they are defined by the fact that they convey characteristic fragrances. It follows that the common tendency to speak of essential oils as a category, as if that implied anything in particular about their medical, pharmacological, or culinary properties, is highly unreliable and often actually dangerous [3].

Ginger, the rhizome of *Zingiber officinale* is one of widely used species of the zingiber family (zingibereace) and is common used for various foods and beverages. Oleoresin (extract crude) has medicine values. The oleoresin of ginger contains the pungent and non-pungent constituents, also in addition to the volatile oil. The major constituent of ginger is lipophilic rhizome extracts, have yielded potentially to active ingredient. The substances which are phenolic ketones are responsible for the pungent flavor of fresh ginger and known as; 6, 8, 10, and 12- gingerols are the main pungent constituents of ginger root. During thermal processing or storage, the gingerols may be modified to a series of homologous compounds known as shogaols (8 - shogaol and 10 - shogaol).These gingerols and shogaols had been reported to possess; anti-inflammatory , antioxidatives and anticancer [3,4].5

There is various extraction methods used in the manufacture and extraction of crude oil and the method used is normally dependent on what type of material is being used. So one of the method is a soxhlet extractions and used for isolation of components in the form of soluble compounds. This method converts the volatile liquid into a vapor and then condenses the vapor back into a liquid and is method

of separating mixtures based on differences in their volatilities in a boiling liquid mixture. Distillation is a unit operation or a physical separation process and not a chemical reaction. It is the most popular and effective method use today in producing essential oils [5]. Solvent extraction is the use of solvents, such as petroleum ether, methanol, ethanol, or hexane, to extract the odoriferous lipophilic material from the plant. The solvent will also pull out the chlorophyll and other plant tissue, resulting in a highly colored or thick/viscous extract. The first product made via solvent extraction is known as a concrete. A concrete is the concentrated extract that contains the waxes and/or fats as well as the odoriferous material from the plant (Li 2009).

There are many factors that can manipulate in the extraction process. One of the factors is solvent extraction. Solvent extraction is usually used to recover a component from either a solid or liquid. The sample is contacted with a solvent that will dissolve the solutes of interest. So it is related to the solubility and viscosity of the solvent itself in order to extract the materials. The other factor is extraction time which has its own effect on the extraction where increasing of extraction time will increase the extracts of the oil. And also the other factor which has been considered in this research is the particle size. The particle size contributes on the yield of oil where it is suggested concentration of essential oil in ginger will decrease when there is increase in size [6].This research purpose is to determine the optimum solid solvent ratio, the extraction time and the particle size to produce high amount of crude oils rhizome of ginger yield [7].

To control several variables in an experiment is challenging upon acquiring a maximum and quality optimum response. Design expert software is a statistical tool approaching controllable variables solid solvent ratio (1:8 ; 1:10 and1:12) by (w/v) , an extraction time (1 hr, 2 hr and 3 hr) and the particle size (1µm ,3µm and 5µm) and responses variables is yield of extract crude oil from ginger allowing detection of possible interaction in a well designated structured and effective experiment plan. Response Surface Methodology (RSM) with Central Composite Design (CCD) is suitable and widely used method in optimization process of essential oil successfully [9, 10].The objective of this study was to analyze the effects of the process parameters on crude oil production from ginger and determine the optimal values for attaining a higher crude oil yield and also characterization of chemical composition of extract crude ginger oil [10].

1.2 Statement of the problem

The demand projection in Ethiopia divulges that the domestic demands for essential oils are substantially increasing with time. This increasing demand of essential oil, such as ginger oil has opened up wide opportunities for global and local market and this leads to the requirement of competitive product in market which comes with all the advantages in terms of cost, quality and its production time. Therefore, it is important to identify an optimum extraction technique, so that a higher quality of a crude oil with higher yield can be extracted.

The demand and supply for oleoresin and essential oils in Ethiopia obtain through import. The worldwide market for essential oils has been estimated at $2.6 billion, with an annual growth rate of 7.5 percent. On average, in Ethiopia more than 6 million US dollar has been allocated every year to import the essential oil (CIA, 2010). This presents the existence of huge demand for the product and the burden it is exerting on the country‘s foreign exchange. Therefore, the rational of this research work is to save the foreign currency and possesses wide range of economic and social benefits such as increasing the level of investment, tax revenue, create job opportunity for urban and rural people, encourage the farmers to produce the plant of ginger and more beneficiary from the product and substitute the import and also support the establishment of new pharmaceutical industry in Ethiopia which contribute for the development of the country. Moreover, the herbal industry is expected to be the main contributor to the country’s income in the future. The rationale of this research work is looking for optimum techniques for the production of ginger oil through which income of farmers improved, job opportunity enhanced and import substitution also diversified.

1.3. Objective

1.3.1 General Objective

The main objective of this study was to optimize and characterize the extract crude oil from Rhizome *Zingiber Officinale* (ginger) and evaluate its microbiological effect.

1.3.2 Specific Objectives

- Extraction of ginger oil by using soxhelt apparatus
- To investigate the optimal parameters of the extraction processes such as solvent ratio, residence time and particle size
- To characterize the chemical components of extract crude ginger oil
- To evaluate its effect on antimicrobial of ginger oil

1.4. The Significance of the study

The significance of extraction of crude oil from Rhizome *Zingiber officinale* (ginger) can be Seen from different perspectives:-

- Provide optimal extraction method for the production of crude extract oil from ginger
- Provide an economically feasible option to produce extract crude oils (ginger oil) locally, which will play a major role to substitute the imported synthetic preservative, additives and flavor liquor which extract from ginger and save hard currency and create job opportunity.
- To assess the potential of new industrial raw material for the extraction of ginger oils.
- It will create an opportunity to develop the industry-university or research institutes linkage to conduct applicable researches which will benefit both the researcher and the industry.
- From the output of the project investors, farmers, and researchers and higher learning institution will be benefited.

CHAPTER TWO

2. Literature Review

2.1 Herbal plants

Medicinal plants have been found as important contributors to the pharmaceutical, agriculture and food industries. With the onset of the synthetic era, pharmaceutical industries are producing a lot of synthetic drugs that help to alleviate the chronic diseases. However, through time many problems associated with frequent use of synthetic drugs become prominent like severe side effects and resistance of microbes against these drugs. On the other side synthetic drugs are expensive and a large population cannot afford these drugs. In recent times research on medicinal plants has been intensified all over the world. The natural pharmaceuticals are receiving extra ordinary importance and popularity as safe, efficacious and cost effective medicines with extraordinary benefits due to combination of medicinal ingredients with vitamins and minerals [11].

Recently there is an emerging trend in research to support the biological activities of medicinal plants. Many scientific researches have been reported about the efficacious and chemotherapeutic role of medicinal plants in the treatment of diverse diseases [12]. Cancer is one of such field where scientists are expecting new compounds from herbs that can provide an important tool for fighting against this dreaded disease. Terminalia arjuna, ginger and flavonoids extracted from different sources have shown significant inhibiting effect on cancer cells [13]. Diabetes mellitus is another area for herbal research, as large number of the population in developing countries is suffering from this problem. Many plants showed tremendous hypoglycemic potential. Trigonella foenum, Allium cepa, Allium sativum and Eugenia jambolan are some famous hypoglycemic plants [14, 15].

Cardiovascular diseases have been become the number one cause of human deaths throughout the world can be controlled by herbal medicines. Many immunomodulatory agents are of plant origin [16]. Hepatic and arthritis are painful diseases and no satisfactory cure of these diseases is present in modern medicines. Many plants have shown their marvelous capability to lower the raised level of liver enzymes in viral hepatitis [17]. Many plants have shown immense potential as anti-peptic ulcer, antimicrobial and antioxidant properties. With widespread the interest in the research of the herbal medicines; these have become an alternate health care system to solve the health problems of world in today's synthetic allopathic era [18, 19]. Pakistan is blessed with rich herbal sources which are being used for medicinal and aromatic purposes. The proper medicinal uses of some of plants are well known, and many have still to be explored.

Pakistan has a wealth of 5700 species of medicinal plants, 456 plants are used to manufacture more than 350 classical formulations to treat many ailments Pakistan is a developing country; there is a need

to facilitate the herbal research and its application to solve the problem of health seeking population[20].

With the advancement of research in medicine, it was concluded that plants are biosynthetic laboratories for chemical compounds, which are responsible for curative action of plants. Scientists isolate phyotochemical from medicinal plants and many of them are found very active against many diseases. A conitine, atisine, nicotine, atropine,and morphineare some famous examples of such phytochemical [21].

2.2 Ginger Rhizome

2.2.1 History of *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. As the rhizome of *Zingiber officinale* Rosc., a perennial herb, of family Zingiberaceae, probably native to southeastern Asia, it is produced everywhere and picked and dug in Autumn and Winter. Its generic name Zingiber is derived from the Greek zingiberis, which comes from the Sanskrit name of the spice, singabera. 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. 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 culinary. 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 [22].

Its use in India and China has been known from ancient times, and by the 1st century AD traders had taken ginger into the Mediterranean region. By the 11th century it was well known in England. The Spaniards brought it to the West Indies and Mexico soon after the conquest and by 1547 ginger was being exported from Santiago to Spain [23].

The leafy stems of ginger grow about a meter high. The leaves are 15 to 30 cm long, elongate, alternate in two vertical rows, and arise from sheaths enwrapping the stem. The flowers are in dense, cone like spikes about 2 cm thick and 4 to 6 cm long composed of overlapping green bracts, which may be edged

with yellow. Ginger is propagated by planting rootstalk cuttings and has been under this type of cultivation for so long that it no longer goes to seed. Harvesting is done simply by lifting the rhizomes from the soil, cleansing them, and drying them in the sun. The dried ginger rhizomes are irregular in shape and branched [24].

The fresh rhizome, green ginger, is used in cooking. The peeled rhizomes may be preserved by boiling in syrup. In elsewhere, slices of ginger are eaten between dishes or courses to clear the palate. Ginger is used medically to treat flatulence and colic. Their color varies from dark yellow through light brown to pale buff. Ginger may be uncrated (with its entire cork layer); partly scraped or scraped or peeled and the root of *Zingiber officinale* shown in Fig.2.1.

Fig. 2.1 Root of Zingiber officinale Rosc.

China is said to be the native home of ginger, and as such, the Chinese are well versed in its ability to sort out problem stomachs. In China, unlike the West, traditional medicine, which is herbalism, never fell out of favor. There, herbal medicine is a fine and sophisticated while others use the whole ginger root [25].

The Chinese, on the other hand, use ginger's papery brown skin to treat people with gas. They bruise and then juice the leaves, using the resulting liquid to increase the appetite of people with no taste for food, and they use the peeled root to treat nausea, dysentery, and to act as an overall digestive stimulant.

After all, the Chinese have been working with ginger as medicine for some 4000 years; it is only reasonable that they should know it a bit better than we should. Ginger was first grown in the Caribbean and Latin America in the late 1500s, and the creeping plant has since become a mainstay in

the practice of local herbalists. In Mexico, the fresh root is grated, mixed with water, and taken after meals to ensure good digestion. In Trinidad, the root is made into tea to treat indigestion and morning

sickness. In Brazil, it is used to treat cramps, nausea, and gas. The story is basically the same around the world: whenever intestinal flu sets in, the symptoms are best treated with ginger [26].

It is also known by various names such as African ginger, black ginger, sunthi, East Indian pepper, Jamica pepper, Germaning wer, Italian zenzerjengibre, myoga, zangvil, gingembre, dinnsear, engifer,shouga, imbir, luya and gung and the taxonomy of ginger shown in Table 2.1 [27].

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

Kingdom	Paltae
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*

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. Ginger flowers have also been described as being greenish yellow and streaked with purple down the sides and the plant of ginger shown in Fig.2.2 [28].

Fig. 2.2 Plant of Ginger (*Zingiber Officinale* Roscoe)

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, 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 [29].

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. In Ethiopia, ginger is produced around southern part of Ethiopia and western part of Oromiya region.

Production of Ginger in Ethiopia

The history of spices in Ethiopia is an ancient one and spices remain as basic food items of the Ethiopian people. Ethiopia has a world share of 0.4 %.(Fact fish). Ethiopia is the homeland for many spices, for example Korarima, long pepper, black cumin, Bishops weed and coriander. The average land covering by spices is approximately 222,700 ha and the production 244,000 ton/annum. At the moment, there are two spice extraction plants in Ethiopia, one public and the other under private ownership. The public spice extraction plant, the Ethiopian Spice Extraction Factory, has a processing capacity of 180 tons per year. The plant is capable of processing ginger from locally grown ginger root, capsicum oleoresin from red pepper, and turmeric. Over 85% of its business is for paprika (ibid, p.6). The privately owned spice extraction plant in Ethiopia is Kassk Spices and Herbs Extraction Plc. This factory was built in Addis Ababa in 1997 and has a processing capacity of 120 tons per annum. All of the extracted spice is exported overseas for food coloring, flavoring, etc. to Europe mainly Germany, Spain and Italy.

Ginger cultivation in Ethiopia started during 13th century. Arabs introduced it from India. Ethiopia is the gate way for many Asian and many East European Countries. Ginger cultivated in many places in Ethiopia. As a point of reference, the Table 2.2 shows the production status the southern National and Nationalities Peoples Regional States, which is the major producer of spices in the country.

Table 2.2 Production status of spices in Ethiopia in tone per annual

Type	Production year			
	2009/10	2010/11	2011/12	2012/13
Ginger	231000	131157	224720	300000
Tumeric	36460	19627	64155	25000
Black Carda	55930	67800	29750	10000
Pepper	110000	51200	29100	150000
Others	1410	9430	7242	15000

Source: The federal democratic republic of Ethiopia central statistics agency (2014).

2.2.2 Main Chemical Compound in *Zingiber Officinale*

Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, is a popular spice used globally especially in most of the Asian countries. Chemical analysis of ginger shows that it contains over 400 different compounds. The major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic compounds [10]. Terpene components of ginger include zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α-curcumene, while phenolic compounds include gingerol, paradols, and shogaol. These gingerols (23–25%) and shogaol (18–25%) are found in higher quantity than others. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also present [30, 31].

Zingiberene is a monocyclic sesquiterpene that is the predominant constituent of the oil of *Zingiber officinale* from which it gets its name. Ginger (*Zingiber officinale*) is a widely used herb and a food - flavoring agent. Its neutraceutical properties have long been an interest to the food processing and pharmaceutical industries. The rhizome of ginger is used as a food ingredient, as well as a traditional medicinal herb to treat many diseases, including gastrointestinal, stomachic, rheumatic disorders and muscular discomfort. The volatile essential oils from ginger extract contribute to the characteristic of

flavor, varies from 1.0-3.0%. However the oleoresin, which responsible for the pungent smell of ginger, Varies from 4.0 – 7.5 % and also possesses substantial antioxidant activity [32].

Among the representative bioactive compounds in ginger, most of them are known as homologous phenolic ketones and exist as 6, 8, and 10- gingerols with different lengths of their unbranched alkyl chains. According to previous researches the gingerols have prominent cancer preventive effects against gastric and colon cancer in vitro and skin cancer in vivo. Several studies revealed that the 6-gingerol has been found to possess various biological activities and pharmacological effects, including anti-inflammatory, analgesic, antipyretic, chemo preventive, angiogenesis, and antioxidant properties [33].

The aromatic constituents include zingiberene and bisabolene, while the pungent constituents are known as gingerols and shogaols. Other gingerol- or shogaol-related compounds (1–10%), which have been reported in ginger rhizome, include 6-paradol, 1-dehydrogingerdione, 6- gingerdione and 10-gingerdione,4- gingerdiol, 6-gingerdiol, 8-gingerdiol, and 10-gingerdiol, and diarylheptanoids [34, 35].The characteristic odor and flavor of ginger are due to a mixture of volatile oils like shogaols and gingerols.

2.2.3 Plant Polyphenols

Polyphenols are widely distributed and important class of plant secondary metabolites, which possess aromatic ring with one or more hydroxyl substituent. Phenolic compounds are mostly water soluble since they are most frequently occurring in combination with sugars as glycosides. Plant polyphenols are very important for growth development and play key role in defense against microbial activities, and infections. They provide oxidative stabilities to the plants in case of injuries [36]. Currently, polyphenols have attracted great attention and get a high importance due to their antioxidant activity. Pharmacological activities of many plants, fruits and vegetables are closely related to the presence of natural antioxidants especially, phenolic acids and flavonoids [Kumar et al., 2007]. These compounds have great importance for their ability to prevent oxidation and used as major ingredients in foods for preservation. Antioxidants significantly decrease the adverse effect of reactive species and at the same time antioxidant therapy has great impact in the treatment of many other diseases [37].Phenolics are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, *etc.*) and beverages (tea, coffee, beer, wine, *etc.*), and partially responsible for the overall organoleptic properties of plant foods. For example, phenolics contribute to the bitterness and astringency of fruit and fruit juices, because of the interaction between phenolics, mainly procyanidin, and the glycoprotein in saliva. Anthocyanins, one of the six subgroups of a large group of plant polyphenol constituents known as flavonoids, are responsible for the orange, red, blue and purple colors of many fruits and vegetables such as apples, berries, beets and onions. It is known that phenolics are the most important compounds

affecting flavor and color difference among white, pink and red wines; they react with oxygen and are critical to the preservation, maturation and aging of the wine [38].

2.2.3.1 Type of plant polyphenols

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors. They are ubiquitous in all plant organs and are therefore an integral part of the human diet

Flavonoids are the most abundant polyphenols in our diets. Flavonoid is themselves divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins, according to the oxygenation or hydroxylation of the central C ring. Their structural variation in each subgroup is partly due to the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation. Some of the most common flavonoids include quercetin, a flavonol abundant in onion, broccoli, and apple; catechin, a flavanol found in tea and several fruits; naringenin, the main flavanone in grapefruit; cyanidin-glycoside, an anthocyanin abundant in berry fruits (black currant, raspberry, blackberry, *etc.*) and daidzein, genistein and glycitein, the main isoflavones in soybean. phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall [39].

Tannins are another major group of polyphenols in our diets and usually subdivided into two groups: hydrolysable tannins and condensed tannins. Hydrolysable tannins are compounds containing a central core of glucose or another polyol esterified with gallic acid, also called gallotannins, or with hexahydroxydiphenic acid, also called ellagitannins [40]. The great variety in the structure of these compounds is due to the many possibilities in forming oxidative linkage. Intermolecular oxidation reactions give rise to many oligomeric compounds having a molecular weight between 2,000 and 5,000 Daltons. Condensed tannins are oligomers or polymers of flavan-3-ol linked through an interflavan carbon bond [41].They are also referred to as proanthocyanidins because they are decomposed to anthocyanidins through acid-catalyzed oxidation reaction upon heating in acidic alcohol solutions. The structure diversity is a result of the variation in hydroxylation pattern, stereochemistry at the three chiral centers, and the location and type of interflavan linkage, as well as the degree and pattern of methoxylation, glycosylation and galloylation [42].

Despite their wide distribution, the health effect of dietary polyphenols has come to the attention of the

nutritionists only in recent years. Researchers and food manufacturers have become more interested in polyphenols due to their potent antioxidant properties, their abundance in the diet, and their credible effects in the prevention of various oxidative stress associated diseases [43].

The preventive effects of these second plant metabolites in terms of cardiovascular, neurodegenerative diseases and cancer are deduced from epidemiologic data as well as in vitro and in vivo and result in respective nutritional recommendations [44]. Furthermore, polyphenols were found to modulate the activity of a wide range of enzyme and cell receptors. In this way, in addition to having antioxidant properties, polyphenols have several other specific biological actions in preventing and or treating diseases [45].

2.2.3.2 Bioactivities of Polyphenols

There has been an upsurge of attraction in the remedial potential of medicinal plants in free radical associated diseases. Polyphenols are widely distributed secondary metabolites and have been reported to exert multifarious biological effects, including anti- infectious, antioxidant, cardio protective, immunomodulatory and anti- carcinogenic [46].

- **Polyphenols as Antioxidants**

Free radicals are reactive species generated in the body as a result of many metabolic pathways like respiration and cell mediated immune functions. Free radicals are also introduced through exogenous sources such as environmental pollution, pesticides and exposure to radiations They are categorized as Reactive oxygen species (ROS), including free radicals like super oxide anion(O_2^-), Hydroxyl radical ($OH\cdot$), and non-radical species like hydrogen peroxide (H_2O_2) and singlet oxygen. Reactive nitrogen species (RNS) including NO ·, $NO_2\cdot$ as free radicals and HNO_2, N_2O_4 as non-radicals. Different environmental factors and aging elevate the level of free radicals and cells become unable to work efficiently against the free radicals leading to accumulation of radicals and oxidative stress which results in cellular damage [47]. ROS and RNS deteriorate many biological molecules like fatty acid, lipids proteins and DNA, and become a major cause of heart diseases, diabetes, cancer, inflammations and weak immune system Nature has gifted the defense system to protect the body from injurious effects of free radicals. This includes enzymatic defense systems like superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) and the non-enzymatic are vitamins (A, C, E) and polyphenols.

All of these act as antioxidants and maintain the level of free radicals in the body. Their mode of action varies and the most common are; reduction, scavenging of frees radicals and singlet oxygen and

formation of complexes with pro oxidant metals. The balance between antioxidants and oxidation is believed to be very essential for healthy biological system.

From the last few years interest in studying and quantifying the antioxidant components of fruits, vegetable and medicinal plants has been increased due to their potential health benefits. Phenolic antioxidants stop oxidation in the food system as well as in the human body and defend them from the detrimental effects of free radicals. Polyphenols are the bounteous antioxidants in the diet. Their total consumption as diet could be much higher than other groups of phytochemicals and recognized nutritional antioxidants like vitamin C, E and carotenoids. Polyphenols as source of antioxidants play an incredibly imperative role to the prevention of cardiovascular diseases, cancers, osteoporosis, neurodegenerative diseases and diabetes mellitus [48, 49].

- **Antioxidant Mechanism of Polyphenols**

There are possibilities of more than one antioxidant mechanism of polyphenols but it is strongly suggested that polyphenols show their antioxidant action by scavenging free radical via hydroxyl group of phenols and their reactivity attributed to phenolic moiety Phenolic compounds generally donate their hydrogen atom to reduce reactive species and itself phenolic compounds are converted into phenoxy radical (ArO ·), which get resonance stability due to delocalization of unpaired electron over aromatic ring furthermore which change into quinones.

$ROO\cdot + ArOH \longrightarrow ROOH + ArO\cdot$ …… (2.1)

$HO\cdot + ArOH \longrightarrow HOH + ArO\cdot$ …… (2.2)

Phenoxy radical (ArO·) intermediates are stable and inhibited further oxidation reaction. Phenolic compounds also regenerate the oxidized form of other antioxidant like vitamin C and they work synergistically with other antioxidants such as E and C. Phenolic compounds may also impart antioxidant properties by functioning as chelators of metal ions that are capable of catalyzing oxidation [50].

- **Determination of Antioxidant Activity**

In vitro antioxidant analysis of phenolic compounds depends upon their free radical scavenging potential. In vitro analysis often uses chemicals and reagents to generate free radicals so that the radical scavenging ability of the test antioxidant can be determined. Several methods have been developed in which the antioxidant activity is assessed by the scavenging of synthetic radicals in polar organic solvents.

Most commonly used synthetic free radicals are 1-1-diphenyl 2 picrylhydrazyl (DPPH) and 2, 2

Azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS). In these assays the neutralization of stable radicals with an antioxidant is measured colorimetrically, which is proportional to concentration of free radical scavengers. Reduction of NBT is the most important method to evaluate the super oxide scavenging activity. Super oxide radical may be formed by different ways; one of these is oxidation of hydroxylamine. The superoxide anion reduces NBT into color compound which can be measured colorimetrically at 560 nm. Scavenging of nitric oxide free radical is an important parameter to measure antioxidant activity because it is another very reactive free radical. Nitric oxide is produced from sodium nitro prusside which forms a color complex with greiss reagent. The absorbance of chromophore with or without scavenger is evaluated at 546 nm .A significant numbers of studies are reported regarding the phenolics contents and antioxidant activity of various medicinal plants. Antioxidant with remarkable antioxidant activity has been found in many medicinal plants studied the relationship between structural characteristic of flavonoids with their antiradical activity. Results of this study revealed that free radical quenching activity of polyphenolic compounds strongly depends on the specific substitution pattern of free hydroxyl moieties on flavonoids structure. Many studies reported that there is a linear relationship between the phenolic contents and antioxidant activity evaluated 32 herbs of Poland for antioxidant activity and phenolic contents. A positive correlation was found between total Polyphenolic contents and antioxidant activity. All extracts showed good correlation (R = 0.92) with total polyphenols and inhibition of DPPH, ABTS radicals and iron reduction [51, 52].

2.2.3.3 Antimicrobial Activity

Infectious disease resulting from the presence of pathogenic microbial agents including bacteria, fungi, and viruses has become a major healthcare problem in current century. Infectious diseases are the main reason of deaths in developing countries .Incidence of new and re-emerging infectious diseases and development of resistance to antibiotic is alarmingly increasing. In modern time treatment of infectious diseases becomes a big problem due to the side effects of some antibiotics which includes hypersensitivity allergic reaction and immunosuppression. There is need of time to discover new antimicrobial compounds with different chemical structures and novel mechanism of action. Diverse antibiotics of synthetic and microbial origins have been produced.

Indiscriminate use of antimicrobial drugs has created very dangerous drug resistance to microbial strains; many bacterial strains have developed resistance against antibiotics, such as penicillin resistant *Streptococcus pnemoniae*, methicillin resistant *Staphylococcus aureus*. Due to the development of bacterial super resistant strains currently used antibiotic failed to cure the infectious diseases .Solution of antibiotic resistance is the development of new drugs from synthetic or natural sources. Therefore

discovery of new antibiotic sources that can act either by direct antimicrobial activity or by preventing resistance of microorganism with minimal side effects is emerging and is of paramount need .However previous records showed that even new families of synthetic antimicrobial agent will have short life expectancy. Researchers turned their attention towards herbal products, which is most promising area in search of new biologically activity compounds with better activity against multi drug resistant strains and reduced antibiotic related side effects[53, 54].

Antimicrobial potential of some plants had been accepted long before mankind discovered the presence of microbes. The healing power of plants is usually due to presence of secondary metabolites. Plant extracts and large number of phytochemicals exhibited strong inhibiting effect on a broad spectrum of microorganisms (fungi, bacteria). Beside bacterial infections fungal infections are also a big threat to the life of the human beings. Only few antifungal drugs are available and long use of these drugs caused resistance.Plants produce a great variety of chemical compound as in their defense system these defense molecules are secondary metabolites. Plants are rich in secondary metabolites such as tannins, alkaloids and flavones, which have been shown antimicrobial properties [55].

2.2.4 Benefit of ginger rhizome

Ginger is one of the most ancient spices in worldwide cuisine. It has become well-known for its various health benefits, which include its ability to boost bone health, strengthen the immune system, increase appetite, prevents various types of cancer, improve respiratory conditions, aid digestion, eliminates arthritis symptoms, reduce excess gas, enhance sexual activity, and relieve pains related to menstrual disorders, nausea, and flu.

The scientific literature provides evidence that ginger has a number of potential health benefits. This evidence suggests that ginger may help to alleviate nausea, both during pregnancy and from other causes. Some research suggests positive benefits of ginger in alleviating inflammation, especially that contributing to osteoarthritis. Preliminary evidence is also available on ginger and relief of hypertension and that ginger intake may have a role in cancer prevention. Finally, initial preclinical research demonstrates that ginger lowers blood cholesterol and blood glucose levels. In general, the preclinical data and preliminary findings suggest a variety of potential health benefits of ginger, although clinical trials supporting these benefits are relatively few [56].

The rhizome contains fats, carbohydrates, protein, fiber, water, and volatile oil. The quality and quantity of biologically active constituents of ginger depend on its cultivation practices and postharvest treatment. The chemical components of the ginger rhizome can vary considerably, depending on the location of cultivation and whether the product is fresh, dried, or processed. The

pungency of fresh ginger results from a group of phenols, the gingerols, of which 6 -gingerol is most abundant. Fresh ginger also may contain a 5-deoxy derivative of ginger called paradol. Dry ginger, on the other hand, exhibits pungency due to the shogaols, which are dehydrated forms of gingerols resulting from thermal processing. Ginger also contains about 1% to 3% volatile oil that imparts a distinctive odor to ginger and which is composed mainly of monoterpenoids and sesquiterpenoids, including camphene, borneol, zingiberene, Sesquiphellandren bisabolene.Besides the pungent phenolic compounds (gingerols and shogaols), there are also bioactive diarylheptanoids and zingerone that are believed to contribute to its purported health benefits [57].

Traditional Use of Ginger is cultivated for millennia in both China and India; ginger reached the West at least 2,000 years ago. Most of the thousands of prescriptions in Chinese traditional medicine (TCM) are combinations of many herbs; ginger is used in nearly half of them to mediate the effects of other ingredients as well as to stimulate the appetite and calm the stomach [Megan Ware RDN LD]. In European herbal traditions, ginger is primarily used to stop nausea and quiet an upset stomach. And also the ginger root is used as nutrition. The nutritional value of ginger root is shown below Table2.3.

Table 2.3 Nutrient value of fresh ginger root (*Zingiber officinale*)

Principle	**Nutrient Value/100g**	**Percentage of RDA (%)**
Energy	80Kcal	4
Carbohydrate	17.77g	13.5
Protein	1.82g	3
Total Fat	0.75g	3
Dietary Fiber	2.0g	5
Vitamins		
Folates	11 µg	3
Niacin	0.75 mg	4.5
Pantothenic acid	0.203	4
Pyridoxine	0.16 mg	12
Vitamin C	5 mg	8
Vitamin E	0.26 mg	1.5
Vitamin K	0.1 µg	0
Electrolytes		
Sodium	13 mg	1
Potassium	415 mg	9

Minerals		
Calcium	16 mg	1.6
Copper	0.226 mg	25
Iron	0.60 mg	7.5
Manganese	0.229 mg	10
Magnesium	0.229 mg	11
Zinc	0.34 mg	3

Source: USDA National Nutrient data base (2016)

Current Uses of Ginger

Ginger also has been found to increase gastric juice secretion and the production of hypo chloride. This means that food is digested more quickly; creating an unfriendly environment for bacteria that could end up in diarrhea. Along these lines, chemicals in ginger have been proven to knock out the sort of bacteria that cause 'Delhi belly' and 'Montezuma's revenge'. One of the classic treatments for bacterial dysentery in the tropics is ginger, and people there are well advised to use this cheap and effective cure. The key to ginger's use in cases of intestinal flu due to bacteria, and indeed in cases of food poisoning, may lie in its high content of volatile oil. The root may contain as much as three percent volatile oil, which is a lot for a plant. Volatile oils have a powerful bacteria-killing capacity, and it seems probable that as the volatile oil floats down the digestive tract, it kills bacteria along the way. Typical illnesses treated with ginger include bacterial dysentery, cholera, diarrhea, nausea, vomiting, chills, cramps, and lack of appetite [58].

Ginger, Zingiber officinale, is inaccurately referred to as “ginger root”, although the edible section sold in the markets and used in dishes, is actually the stem or the rhizome. In Western cultures, it is mostly used in sweets and alcoholic beverages such as ginger beer and ginger wine. However, in Asian cultures, ginger is directly used by chopping it up or using its powder in traditional dishes and in soft drinks such as coffee and tea. Ginger’s irresistible fragrance is due to an essential oil in its composition that has been coveted and extracted by perfume makers since ancient times. Not only is ginger known as an essence and a spice, it is known to be one of the oldest remedies known in herbal and aromatic traditional treatments, especially in China,

India, and the Middle East. In China, it has been used for over 2,000 years for curing inflammation and diarrhea. Ginger truly does top the list of effective natural home remedies. Being used throughout history by different cultures around the world, ginger harnesses an incredible healing power proven for a host of ailments. The spice is packed with essential nutrients and rejuvenating compounds. While

ginger has been shown to help countless ‘minor’ problems such as an upset stomach, amazingly the health benefits of ginger also include combating cancer more effectively than pharmaceutical cancer drugs [59].

2.3 Essential oils

An essential oil is a volatile material derived from a plant, and it usually bears the aroma or flavor of that plant. Although a few animal-derived aromatic products exist (mainly musk, civet, and ambergris), the ones of botanical origin are far more numerous. Like fixed oils (vegetable oil, motor oil), these substances are generally liquids, they won’t mix with water, and they are soluble in many organic solvents. Unlike fixed oils, however, essential oils are volatile: they evaporate rapidly at room temperature, whereas fixed oils will not. Chemically, an essential oil is a complex mixture of 30 to 100 or more compounds. Only with the advent of modern analytical techniques, particularly gas chromatography, have we fully appreciated the complexity of these mixtures. With gas chromatography, an oil is separated into its components, and the relative proportions of the components are represented graphically as a series of peaks; some large, some small. The area under each peak represents the proportion of each component in the oil, and by experience, structural analysis, and comparison of the chromatogram with others made with pure reference chemicals, we can identify many of the components. Next time you touch and sniff an herb, remember that your nose is being bombarded by a wide array of chemicals [60].

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 [61]. 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 [62].

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the oil of the

plant from which they were extracted, such as oil of clove. Oil is "essential" in the sense that it contains the "essence of" the plant's fragrance, the characteristic fragrance of the plant from which it is derived. The term essential used here does not mean indispensable as with the terms essential amino acid or essential fatty acid which are so called since they are nutritionally required by a given living organism. Essential oils contain the true essence of the plant it was derived from. Essential oils are highly concentrated and a little goes a long way.

An essential oil is a liquid that is generally distilled (most frequently by steam or water) from the leaves, stems, flowers, bark, roots, or other elements of a plant. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, and cold pressing. They are used in perfumes, cosmetics, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning product. Essential oils, contrary to the use of the word "oil" are not really oily-feeling at all. Most essential oils are clear, but some oils such as patchouli, orange and lemongrass are amber or yellow in color.

Essential oils are volatile and liquid aroma compounds from natural sources, usually plants. It is also highly concentrated essences of aromatic plants. Essential oils are not oils in a strict sense, but often share with oils a poor solubility in water. The plants extracts are assumed to be more acceptable and for sure they are less hazardous compare to the synthetic compound. Essential oils normally contain a complex mixture of organic compounds and they are largely composed of a range of saturated or partly unsaturated cyclic and linear molecules of relatively low molecular mass, and within this range a variety of hydrocarbons and oxygenated compounds occur [63].

2.3.1 The history of essential oil

Essential oils, or aromatic oils as they were once called, have been used by many cultures around the world for centuries. Their uses varied between cultures from religious purposes to healing the sick. It is difficult to pinpoint exactly when essential oils gained notoriety as effective healing agents, but eventually the knowledge of essential oils spread around the globe. Evidence and recorded history have both shown that the Egyptians used aromatic oils as early as 4500 B.C. They became renowned for their knowledge of cosmetology, ointments and aromatic oils. The most famous of their herbal preparations Kyphi was a mixture of 16 ingredients that could be used as incense, perfume or medicine. They used balsams, perfumed oils, scented barks, resins, spices and aromatic vinegars in everyday life. Oils and pastes from plants were transformed into pills, powders, suppositories, medicinal cakes and ointments.

Ashes and smoke from anise, cedar, onion, garlic, grapes and watermelon among others were also used. At the height of Egypt's power, priests were the only authorities allowed to use aromatic oils, as they were regarded as necessary to be at one with the Gods. Specific fragrances were dedicated to each deity and their statues were anointed with these oils by their followers. Pharaohs had their own special blends for meditation, love, war and so on. Essential oils have been used throughout recorded history for a wide variety of wellness applications. The Egyptians were some of the first people to use aromatic essential oils extensively in medical practice, beauty treatment, food preparation, and in religious ceremony. Frankincense, sandalwood, myrrh and cinnamon were considered very valuable cargo along caravan trade routes and were sometimes exchanged for gold. Borrowing from the Egyptians, the Greeks used essential oils in their practices of therapeutic massage and aromatherapy. The Romans also used aromatic oils to promote health and personal hygiene. Influenced by the Greeks and Romans, as well as Chinese and Indian Ayurvedic use of aromatic herbs, the Persians began to refine distillation methods for extracting essential oils from aromatic plants. Essential oil extracts were used throughout the dark ages in Europe for their anti-bacterial and fragrant properties [Suzanne Bovenizer CMT].

The use of aromatic oils was first recorded in China between 2697-2597 B.C. during the reign of Huang Ti, the legendary Yellow Emperor. His famous book "The Yellow Emperor's Book of Internal Medicine" contains uses for several aromatics and is still considered a useful classic by practitioners of eastern medicine today [https://healingscents.net].

The history of essential oils is intertwined with the history of herbal medicine, which in turn has been an integral part of magical practices. Herbal medicine has been used for more than treating minor ailments and disease; it has been instrumental in providing life-enhancing benefits. In most ancient cultures, people believed plants to be magical, and for thousands of years herbs were used as much for ritual as they were for medicine and food. According to medical herbalist and healer Andrew Chevalier [2007], the presence of herbs in burial tombs attests to their powers beyond medicine. In addition, fourth-century Greek philosopher Aristotle noted his belief that plants had psyches [64, 65].

Aromatic plants in the form of oil and incense were elements of religious and therapeutic practices in early cultures worldwide. In addition, anointment with perfumes and fragrant oils was an almost universal practice. Burning incense in rituals provided a connection between the physical and spiritual between the mundane and the divine. The word perfume comes from the Latin per means through and fume means smoke. It was a common belief that contact with the divine could be achieved through the smoke of incense.

For a time the use of herbs and perfumery were stifled with a double whammy: universities and the emerging medical establishment fought to take herbs out of the hands of the so-called uneducated, and the Christian church steered people away from personal adornment in their bid to hold power over people's lives. As a result, the use of aromatics, even possessing oils and unguents, became a way to identify Witches, and culture again took a backward step. Under Great Britain's King George III, who ruled from 1760 to 1820, a woman's use of scents or potions was equated with seduction and betrayal, and was met with "the same penalties in force against witch craft eventually, herbal practices and perfumery made a comeback as attitudes shifted but by the mid-nineteenth century, essential oils were being replaced by chemicals in medicine. By the twentieth century, perfumes and cosmetics contained mostly synthetic fragrance, which was cheaper and easier to produce. Ironically, a French chemist, Rene-Maurice Gattefosse, was responsible for resurrecting the use of essential oils during the 1920s. After burning his hand in his laboratory, he grabbed the nearest bottle of liquid, which turned out to be lavender oil. Intrigued by the rapid healing effect of the oil, he devoted the remainder of his career to studying essential oils and named his discovery aromatherapy [66].

In brief, aromatherapy is the use of volatile plant oils, including essential oils, for psychological and physical well-being. Although the term aromatherapy was not used until the 20th Century, the foundations of aromatherapy date back thousands of years. The use of essential oils in particular date back nearly one thousand years. The Chinese may have been one of the first cultures to use aromatic plants for well-being. Their practices involved burning incense to help create harmony and balance. Later, the Egyptians invented a rudimentary distillation machine that allowed for the crude extraction of cedar wood oil.

It is also thought by some that Persia and India may have also invented crude distillation machines, but very little is known. Oils of cedar wood, clove, cinnamon, nutmeg and myrrh were used by the Egyptians to embalm the dead. When a tomb was opened in the early 20th century, traces of the herbs were discovered with intact portions of the body. The scent, although faint, was still apparent. Although the cedar wood the Egyptians used was distilled by a crude distillation process, the other oils the Egyptians used were most likely infused oils. The Egyptians also used infused oils and herbal preparations for spiritual, medicinal, fragrant and cosmetic use. It is thought that the Egyptians coined the term perfume, from the Latin per fumum which translates as through the smoke. Egyptian men of the time used fragrance as readily as the women. An interesting method that the men used to fragrance themselves was to place a solid cone of perfume on their heads. It would gradually melt and would cover them in fragrance. The Greeks learned a great deal from the Egyptians, but Greek mythology apparently credits the gift and knowledge of perfumes to the gods. The Greeks also recognized the

medicinal and aromatic benefits of plants. Hippocrates, commonly called the "father of medicine" practiced fumigations for both aromatic and medicinal benefit. A Greek perfumer by the name of Megallus created a perfume called megaleion. Megaleion included myrrh in a fatty-oil base and served several purposes: (1) for its aroma, (2) for its anti-inflammatory properties towards the skin and (3) to heal wounds. The Roman Empire built upon the knowledge of the Egyptians and Greeks. Disco rides wrote a book called De Material Medical that described the properties of approximately 500 plants. It is also reported that Disco rides studied distillation. Distillation during this period, however, focused on extracting aromatic floral waters and not essential oils [Ann Harman, Harvest to Hydrosol Fruitland, WA: botanicals, 2015]. A major event for the distillation of essential oils came with the invention of a coiled cooling pipe in the 11th century. Persian by birth, Avicenna invented a coiled pipe which allowed the plant vapor and steam to cool down more effectively than previous distillers that used a straight cooling pipe. Avicenna's contribution leads to more focus on essential oils and their benefits [67].

Within the 12th century, an Abbess of Germany named Hildegard grew and distilled lavender for its medicinal properties. Within the 13th century, the pharmaceutical industry was born. This event encourages great distillation of essential oils. During the 14th century, the Black Death hit and killed millions of people. Herbal preparations were used extensively to help fight this terrible killer. It is believed that some perfumers may have avoided the plague by their constant contact with the natural aromatics. Within the 15th century, more plants were distilled to create essential oils including frankincense, juniper, rose, sage and rosemary. A growth in the amount of books on herbs and their properties also begins later in the century. Paracelsus, an alchemist, medical doctor and radical thinker is credited with coining the term Essence and his studies radically challenged the nature of alchemy and he focused upon using plants as medicine. During the 16th century, one could begin purchasing oils at an apothecary and many more essential oils were introduced. During the 16th and 17th centuries, perfume starting being considered an art form, and it was more clearly defined as its own field. During the 19th century, perfumery remained a prosperous industry. Women would have their jeweler create a special bottle to hold their treasured perfume. The 19th century also was important scientifically as major constituents of essential oils became isolated. During the 20th century, the knowledge of separating the constituents of essential oils was used to create synthetic chemicals and drugs. It had been believed that by separating the major constituents and then using the constituents alone or in synthetic form would be beneficial therapeutically and economically. These discoveries helped lead to modern medicine and synthetic fragrances. This actually weakened the use of essential oils for medicinal and aromatic benefit. During the earlier part of the 20th century, a French chemist

by the name of Rene-Maurice Gattefosse became interested in the use of essential oils for their medicinal use. Previously, he focused on the aromatic use of essential oils, but his interest in their medicinal use grew further upon using lavender essential oil after an accident. From the late 20th century and on into the 21st century, there is a growing resurgence to utilize more natural products including essential oils for therapeutic, cosmetic and aromatic benefit. The use of essential oils never ceased, but the scientific revolution minimized the popularity and use of essential oils in one's everyday life. Today's heightened awareness regarding the use of synthetics coupled with the increased availability of aromatherapy information within books and the Internet has refueled the use of essential oils for therapeutic, cosmetic, fragrant and spiritual use [68].

2.3.2 The main interest compounds in essential oil

Pure essential oils are mixtures of more than 200 components, normally mixtures of terpenes or phenylpropanic derivatives, in which the chemical and structural differences between compounds are minimal. They can be essentially classified into two groups:

- Volatile fraction: Essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.
- Nonvolatile residue: that comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, and waxes.

It is interesting to know the chemical components that nature combines to make up the oils, but it is also humbling to take note of the fact that even with the best human efforts, should you in a laboratory combine all the chemicals in the correct proportions, you would still not have an identical oil. Such a copy of oil will not have the same therapeutic effect as the natural and pure essential oil. Modern science can still not unlock the secrets of essential oils and why plants produce them. Essential oils, like all organic compounds, are made up of hydrocarbon molecules and can further be classified as terpenes, alcohols, esters, aldehydes, ketones and phenols. Most compounds found in essential oils are terpenoid molecules and they are the carbon backbone and consist of either 10, 15 and rarely with 20 and 25. carbon atoms - made up from the 5 carbon isoprene units.Isoprene is not a terpene, yet all terpenes consist of isoprene units and these terpenes are either found in the plant material from which essential oil is extracted. Essential oils may have two major components which are terpene hydrocarbon and oxygenated compounds. The terpene hydrocarbon can be divided into two group; monoterpenes and sesquiterpenes. While oxygenated compounds are phenols, monoterpenes, and

sesquiterpenes of alcohols, aldehydes, ketons, esters, lactones, coumarins, ethers, and oxides. There are three main aromatic groups which are phenols, terpenes alcohols, and aromatic aldehydes [69].

2.3.3 Usage of essential oils

Essential oils have been used for thousands of years in various cultures for medicinal and health purposes. Essential oil is used in range from aromatherapy, household cleaning products, personal beauty care and natural medicine treatments [70].

The uses for essential oils (both for health and emotions) are vast and diverse. The essential oils that are extracted from plant leaves, flowers, stems, roots, or bark are incredible tools in everyday lives, and when looking for natural and healthy solutions in a more potent form. In addition to these impressive qualities, they often also transfer very pleasurable sensory experiences within minutes because of their fragrances and restorative natures, emotional well-being, and spiritual wellness [71].

Essential oils are used extensively in aromatherapy and various traditional medicinal systems. Due to the numerous health benefits of essential oils, they are increasingly being explored by the scientific community for the treatment of a variety of diseases including cancer, HIV, asthma, bronchitis, heart strokes, and many more[www.organicfacts.net]. There are more than 90 essential oils, and each has its own health benefits. Most essential oil blends well with other essential oils in terms of function and odor, which allows herbalists to prepare a vast repertoire of aromatic essential oil combinations [72].

Essential oils can also have antibacterial or anti-fungal benefits used in medical settings. Many oils when massaged on the skin can heal or help treat skin conditions, such as burns, or cuts and scrapes. Others may help boost the immune system, help with insomnia, and aid with digestion. Technically, essential oils aren’t oils at all, as they lack fatty acids. Rather, they’re highly concentrated plant components

The essential oil has been used in many applications since long time ago. It has been found to be applied traditionally in medications, fragrances, flavors, preservatives and insect repellents. Essential oils often have an odor and used in perfumery. They are actually made up of many different volatile compounds and the makeup of the oils quite often varies between species. The essential oils that are registered food grade materials could be used as alternative anti-fungal and anti-bacterial treatments for fresh produce. The oils are usually extracted by distillation, cold pressing, or extraction method.

2.4 Extraction

Extractions are a way to separate a desired substance when it is mixed with others. The mixture is brought into contact with a solvent in which the substance of interest is soluble, but the other substances are insoluble. Extractions use two immiscible phases to separate the substance from one phase into the other. Typical lab extractions of organic compounds from an aqueous and solid phase into an organic phase. The distribution of a solute between two phases is an equilibrium condition described by partition theory.

Extraction is a technique used to separate compounds based upon their different solubility in two solvents that do not mix. Most commonly, one of the solvents will be water and the other will be an immiscible organic solvent (often methylene chloride, n- hexane, diethyl ether, or ethyl acetate). In general, very non-polar compounds will portion to the organic solvent and very polar compounds and salts will portion to the aqueous phase.

The world production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. The traditional technologies pertaining to essential oil processing are of great significance and are still being used in many parts of the globe. Water distillation, water and steam distillation, steam distillation, cohobation, maceration and enfleurage are the most traditional and commonly used methods. Maceration is adaptable when oil yield from distillation is poor. Distillation methods are good for powdered almonds, rose petals and rose blossoms, whereas solvent extraction is suitable for expensive, delicate and thermally unstable materials like jasmine, tuberose, and hyacinth. Water distillation is the most favored method of production of citronella oil from plant material [73].

Several approaches can be employed to extract the plant material. Although water is used extraction in many traditional protocols, organic solvents of varying polarities are generally selected in modern methods of extraction to exploit a various solubility of plant constituents [74].

2.4.1 Maceration

This simple widely used method involves leaving the pulverized plant soaked in a suitable solvent in a closed container.Simple maceration is performed at room temperature by mixing the ground material with the solvent and leaving the mixture for several days with occasional shaking or stirring. The extraction is then repeated from the plant particles by straining with fresh solvent.

Finally the last residue of extract is pressed out of the plant particles using a mechanical press or a centrifuge.Kinetic maceration differe from simple one by continous stirring.The method is suitable for

both initial and bulk extraction.The main disadvantage of maceration is that the process can be quite time-consuming from a few hours up to several weeks.

2.4.2 Percolation

The powdered plant material is soaked initially in a solvent in a percolator.Additional solvent is then poured on top of the plant material and allowed to percolate slowly (dropwise) out through the bottom of the percolator. Additional filtration of the extract is not required because there is a filter at the outlet of the percolator.Percolation is adequate for both initial and large-scale extraction.The main disadvantages are :

Fine powders and materials such as resins and plants that swell excessively (e.g., those containing mucilages) can clog the percolator, and if the material is not distributed homogenously in the container, the solvent may not reach all areas and the extraction will be incomplete.

2.4.3 Soxhlet Extraction

This method is adequate for both initial and bulk extraction.The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to the flask, and the set up is heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath.The solvent is heated to reflux again and this cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compounds dissolved in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.The main advantage of Soxhlet extraction is that it is a continuous process. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This technique is particularly useful in cases when the pure compound is partially soluble in a solvent and the impurity is not soluble in that solvent and vice versa. Also the working principle of mechanism is so simple that we can obtain more desired compounds without any difficulty. It is the most useful apparatus for solid–liquid extraction in various fields such as pharmaceutics, environment and also foodstuffs nowadays, Sox let apparatus is still common and widely used as a reference and standard method in many laboratories for

the extraction of oil from various materials. The Schematic representations of a Soxhlet extractor shown Fig. 2.3

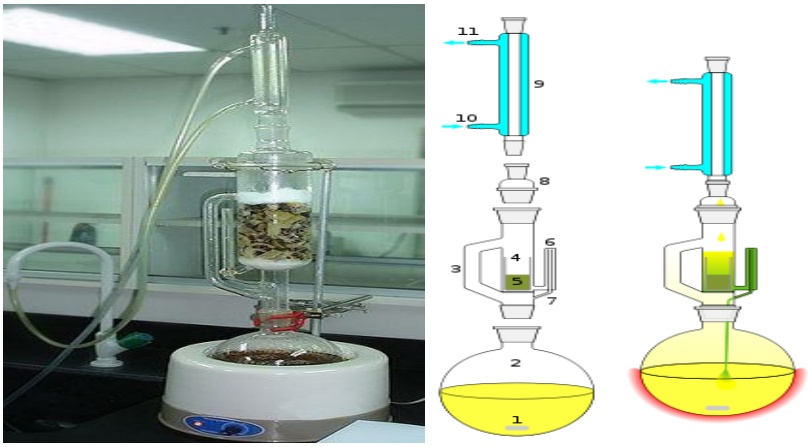


Fig.2.3 Schematic representations of a Soxhlet extractor and Ginger extraction in progress. The sample is placed in the thimble1: Stirrer bar 2: Still pot (the still pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the soxhlet chamber) 3: Distillation path 4: Thimble 5: Solid 6: Siphon top 7: Siphon exit 8: Expansion adapter 9: Condenser 10: Cooling water in 11: Cooling water out

2.4.4 Pressurized Solvent Extraction

Pressurized Fluid Extraction (PFE) or Pressurized Liquid Extraction (PLE) is a new sample extraction method that employs liquid solvents at elevated temperatures and pressures to prepare samples for analysis by either gas chromatography or liquid chromatography. Pressurized liquid extraction is similar to Soxhlet extraction, except that during the extraction process the solvent condition inside the PLE cell approaches the supercritical region which results in more efficient extractions.

The elevated temperature allows the sample to become more soluble and achieve a higher diffusion rate while the elevated pressure keeps the solvent below its boiling point. At elevated pressures and temperatures solvents can penetrate solid samples more efficiently which reduces solvent usage.

The powdered plant material is loaded into an extraction cell, which is placed in an oven. The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurized at programmed levels for a set period of time. The cell is flushed with nitrogen gas, and the extract, which is automatically filtered, is collected in a flask. Fresh solvent is used to rinse the cell and to dissolve the remaining components. A final purge with nitrogen gas is performed to dry the material.Offers a more economical and environment-friendly alternative to conventional approaches.

Pressurized solvent extraction or ‘‘accelerated solvent extraction’’ employs temperatures that are higher than those used in other methods of extraction, and requires high pressures to maintain the solvent in a liquid state at high temperatures. It is best suited for the rapid and reproducible initial extraction of a number of samples. The solid biomass sample is loaded into an extraction cell, which is placed in an oven. The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurized at programmed levels for a set period of time. The cell is flushed with nitrogen gas, and the extract, which is automatically filtered, is collected in a flask. Fresh solvent is used to rinse the cell and to soluble the remaining components. A final purge with nitrogen gas is performed to dry the material. High temperatures and pressures increase the penetration of solvent into the material and improve metabolite solubility, enhancing extraction speed and yield. Moreover, with low solvent requirements, pressurized solvent extraction offers a more economical and environment-friendly alternative to conventional approaches .As the material is dried thoroughly after extraction, it is possible to perform repeated extractions with the same solvent or successive extractions with solvents of increasing polarity. An additional advantage is that the technique can be programmable, which will offer increased reproducibility. However, variable factors, e.g., the optimal extraction temperature, extraction time, and most suitable solvent, have to be determined for each sample.

2.4.5 Hydro distillation

plant material is immersed in a solvent commonly water in a round-bottomed flask, which is connected to a condenser. The solvent is heated until it reaches its boiling point. As the vapor is condensed, the solvent is recycled to the flask.It is commonly applied to the extraction of plant essential oils.The main disadvantage is that thermolabile components risk being degraded.

2.4.6 Extraction with supercritical fluids

Supercritical fluid extraction (SFE) is one of the relatively new efficient separation methods for the extraction of essential oils from different plant materials. The new products, extracts, can be used as a good base for the production of pharmaceutical drugs and additives in the perfume, cosmetic, and food industries. Use of SFE under different conditions can allow selecting the extraction of different constituents. The main reason for the interest in SFE is the possibility of carrying out extractions at temperature near to ambient, thus preventing the substance of interest from incurring in thermal denaturation. This plays a mechanistic role in supercritical fluid chromatography (SFC), where it contributes to the separation of the solutes that are injected into the chromatographic system. Supercritical fluid extraction is an interesting technique for the extraction of flavoring compounds from vegetable material. It can constitute an industrial alternative to solvent extraction and steam

distillation processes. SFE allows a continuous modification of solvent power and selectivity by changing the solvent density.

Nevertheless, the simple SFE process, consisting of supercritical CO_2 extraction and a one-stage subcritical separation, in many cases does not allow a selective extraction because of the simultaneous extraction of many unwanted compounds. Supercritical fluids (SCFs) are increasingly replacing organic solvents,e.g., n-hexane, dichloromethane, chloroform, and so on, that are conventionally used in industrial extraction operations because of regulatory and environmental pressures on hydrocarbon and ozone-depleting emissions. Most of the currently available solvent free extraction systems utilize CO_2, which is generally considered as safe for solvent free extraction processes. The fundamental steps involved in SFE are as follows:

(1) Liquid CO_2 is forced into supercritical state by regulating its temperature and pressure.

(2) Supercritical CO_2 has solvent power and extracts predominantly lipophilic and volatile compounds.

(3) Gaseous CO_2 returns to CO_2 tank. After a full round, the new extraction starts with circulating CO_2 and the flow diagram of SFE is shown in Fig.2.4

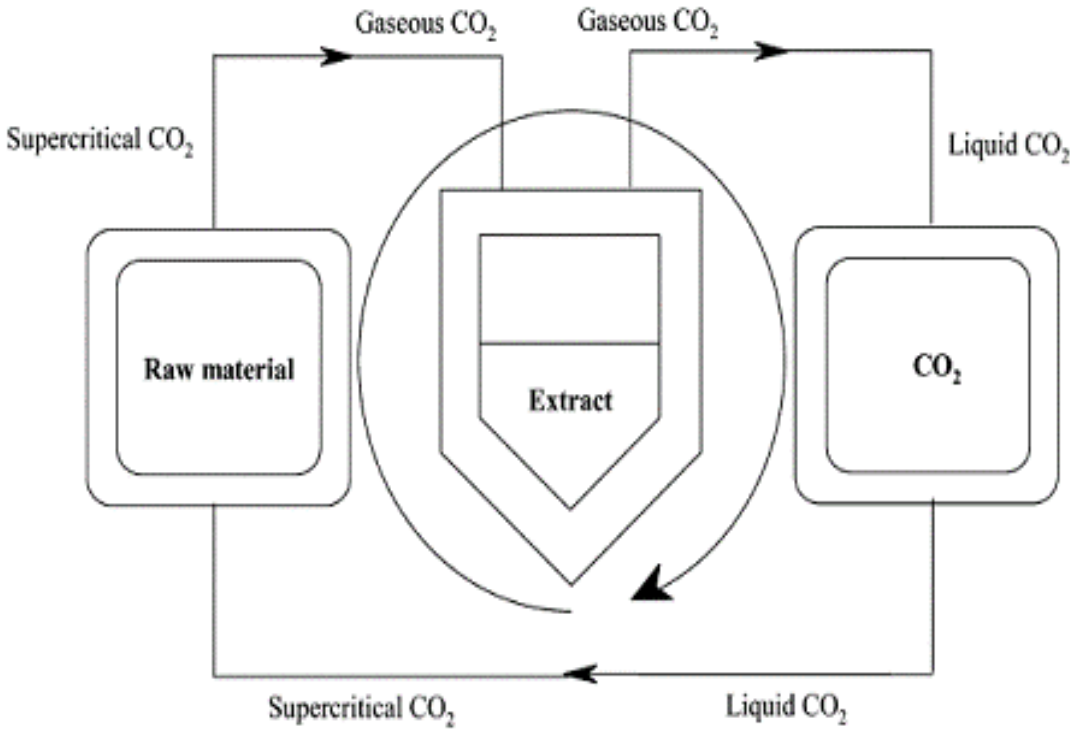


Fig. 2.4 Extraction with supercritical fluids

2.4.7 Microwave Assisted Extraction (MAE)

Microwaves heat up the molecules by dual mechanism of ionic conduction and dipole rotation. Non-ionizing electromagnetic waves positioned between the X-ray and infrared rays in the electromagnetic spectrum with frequency between 300 MHz to 300 GHz are called microwaves. The two types of oscillating perpendicular fields that generate microwaves are the electric field and magnetic field. Both the ionic conduction and dipole rotation are responsible for heating of substances. When the microwaves interact with polar solvents, heating of substance is caused due to any one of the above mentioned phenomena, individually or simultaneously. The electrophoretic migration of ions under the influence of the changing electric field is called ionic conduction. If the solution offers a resistance to this migration of ions, a friction is generated and the solution is heated. The realignment of the dipoles of the molecule with the rapidly changing electric field is called Dipole rotation .At a frequency of 2450 MHz the process of heating occurs. The microwaves have a change in the electric component at a speed of $4.9x10^4$ times/ second. There is a generation of heat through frictional force when the solvent molecules try to align themselves with the changing electric field, but the molecules fail to realign themselves. No heating occurs when the frequency is greater than 2450 MHz and the electrical component changes at a much higher speed .No heating occurs when the frequency is less than 2450 MHz and the electrical component changes at a much lower speed. The inference from the above mentioned mechanisms is that only dielectric material or solvents with permanent dipoles get heated up under microwave .The value of dissipation factor (tan δ), is a measure of the efficiency with which different solvents heat up under microwave[75].

MAE is also one of the extraction methods which can reduces the extraction time and only consumes less volume of solvents and less samples amount. The major disadvantages of this MAE method however is that the solvent needs to be physically removed from the sample matrix upon completion of the extraction prior to further analysis. In the some cases whereby samples are pretreated with activated copper bars to assist the extraction process because the removal of this copper is necessary for a cleaner extract. Although a subsequent purification step can be implemented to rectify this problem, there may be possibilities of losing analytes or inducing contaminants with additional cooling time for this extra handling.

Furthermore, the sample allowance for analysis is limited to 1.0 dg which is insufficient for a homogenous analysis. Microwave-assisted extraction (MAE) or simply microwave extraction is a relatively new extraction technique that combines microwave and traditional solvent extraction. The

microwave energy has been investigated and widely applied in analytical chemistry to accelerate sample digestion, to extract analytes from matrices and in chemical reactions.

Application of microwaves for heating the solvents and plant tissues in extraction process, which increases the kinetic of extraction, is called microwave-assisted extraction. Microwave energy is a non-ionizing radiation that causes molecular motion by migration of ions and rotation of dipoles, without changing the molecular structures if the temperature is not too high. Non polar solvents, such as hexane and toluene, are not affected by microwave energy and, therefore, it is necessary to add polar additives. Microwave-assisted extraction (MAE) is an efficient extraction technique for solid samples which is applicable to thermally stable compounds accepted as a potential and powerful alternative to conventional extraction techniques in the extraction of organic compounds from materials. The microwave-assisted extraction technique offers some advantages over conventional extraction methods.

CHAPTER THREE

3.0 Materials and Methodology

3.1 Materials

The sample used in this study was a fresh ginger (*Zingiber Officinale* Roscoe) which is collected from local market around Addis Ababa and Southern part of Ethiopia. The experimental raw materials were washed with tap water properly and dried under shade. And after drying, the samples were reduced in size with a mill to powder form and ready for the next experiment and the raw material of ginger root is shown in Fig.3.1 and Fig.3.2

Figure 3.1 Sample of ginger root (*Zingiber Officinale* Roscoe)

Figure 3.2 Sample of grinded ginger root

Chemicals: The chemicals that were used for the experiment are chloroform, acetone, dietyl ether, boric acid , potassium iodide (KI) solution, sodium thiosulfate solution, starch solution, distilled water and ethanol, petroleum ether , phenolphthalein indicator, sodium hydroxide (NaOH), Hydro chloric acid (HCl), potassium hydroxide(KOH),sodium bi-carbonate, sodium carbonate, sulphuric acid (H_2SO_4), methanol, n-hexane, all chemicals are analytically graded reagents.

Experimental Location

The experimental works of the thesis had been conducted mainly in different institutions. Size reduction of the raw and extraction process was conducted at the School of Chemical and Bioengineering, Extraction and Optimization of crude oil from ginger was conducted at Wood Technology Research Center and Characterization and physico–chemical testing was conducted at the Ethiopia Food, Medicine and Health care Administration and Control Authority (EFMHACA) and Collage of Natural Science department of chemistry.

Equipment /Apparatus/ used

The equipment used to conduct this study are, soxhlet apparatus, kjeldhal digester and analyzer apparatus, Gas Chromatograph Mass Spectrophotometer (GC – MS), Clevenger apparatus, drying trays , slicer, mortar and pestle , What- man number 1 filter paper ,centrifuge, 0.2 micro meter nylon membrane filter, rotary evaporator ,PH meter, razor blade, sieve, desiccators, digital weighing balance, weight bottle, stopper, oven, tong , Erlenmeyer flask, Buchii bottle, vacuum pump, measuring cylinder, ,cotton, beaker, scissor, oven, glove, mask, goggles. The AAs and Soxhlet apparatus shown in Fig.3.3 and Fig 3.4.

Figure 3.3Atomic absorption spectrophotometer (AAs) AA- 6800 Shimadzu.

Fig. 3.4 soxhlet apparatus shooted during experimental process

Fig.3.5 GC – MS and rotary evaporator shooted during experimental session

3.2 Methodology of the Research

3.2.1 Setting Extraction Parameters

For this experiment the independent variables or factors which have a direct effect on the dependent variables or response yield are extraction solvent ratio (n-hexane) with (volume of 160ml, 200ml and 240 ml) for a fixed volume of solid material, particle size (1 µm, 3 µm, 5 µm) and extraction time (1hr, 2hr, 3hr) for actual variable levels. For each factors, an experimental range was adjusted based on the result of literature data. In order to determine optimization conditions for the extraction process a full factorial design on Design Expert software were used. In this study, the temperature was controlled as constant by adjusting at 40 ^{0}c.

3.2.2 Optimization of extract crude oil of ginger by Soxhlet distillation

In order to optimize the parameters of extraction for achieving maximum crude extract oil yield, the experiment was conducted at three different parameter conditions which were solid to solvent ratio (1:8 , 1: 10 and 1: 12 g/ml), extraction time (1hr ,2hr and 3hr) and particle size (1 µm, 3 µm and 5 µm).Soxhlet extraction is actually one of the methods to extract resinoid such as ginger (*Zingiber Officinale* Roscoe).This extraction method use chemical solvents to extract oils by repeated washing or percolation with organic solvents.

Experimental Procedure:

20g of the grounded ginger samples were extracted in 160ml, 200ml and 240ml of extraction solvent (n- hexane) at different particle size 1 µm, 3 µm, 5 µm and by using a condenser to prevent extraction solvent loss from the rounded flask and also the extraction time for 1hr, 2hr and 3hr respectively. In such a way, extraction was performed in duplicate. Finally, the crude extracted resin was cooled at room temperature, filtered and concentrated from extraction solvent by using an equipment of rotary evaporator and weighed to determine the amount or yield of extract crude oil and stored in vials at 4 °C prior to the analysis. Extraction yield for crude oils were calculated by using the following equation.

$$\text{Oil yield (\%)} = \left(\frac{\text{mass of oil extracted}}{\text{Mass of sample}}\right) \times 100\% \quad \ldots\ldots\ldots \quad (3.1)$$

3.2.3 Chemical analysis of ginger root

The ginger roots used for analysis were fresh without any physical defect. Furthermore, the ginger were surface cleaned and washed in running tap water to remove adhering debris after which the samples were air dried for five days under shade and were grinded to fine powder.

Air dried ginger samples were analyzed for chemical composition at the Ethiopian Food, medicine and Health care Administration and control Authority (EFMHACA). Samples were analyzed chemically according to official analytical chemist (AOAC) [76, 77].

Procedure of extraction of essential oil:

300g of the grounded ginger samples was extracted in one liter of water to obtain essential oils by using Clevenger apparatus at constant temperature 40^{o}c. Due to the influence of hot water and steam, the essential oil is freed from the oil glands in the plant tissue. The vapor mixture of water and oil is condensed by indirect cooling with water. From the condenser, distillate flows into a separator, where oil separates automatically from the distillate water.

3.2.3.1 Determination of Crude protein

Crude protein in the residue was determined by the kjeldahl apparatus. This consists of three techniques of analysis namely: Digestion, Distillation and Titration. Distillation was done using Markham Distillation Apparatus which allowed volatile substances such as ammonia to be steam distilled with complete collection of the distillate. 5ml portion of the digest was taken into the body of the apparatus via small funnel aperture. Then 3ml of 40% (w/v) NaOH was added through the same opening with a 5ml pipette. The mixture was steam distilled for 2 minutes in 50ml conical flask containing 10ml of 2% boric acid mixed indicator solution placed at the receiving tips of the condenser. The boric acid plus indicator solution (bromocresol green) changed color from red to green showing that all the ammonia liberated had been trapped.

Furthermore, digestion was done by taking 0.50g of ground dried sample carefully into the kjeldahl digestion tubes to ensure that all samples put in to the bottom of the tubes and was added to 10ml of conc. H_2SO_4 which were set in the appropriate hole of the digestion block heater in a fume cupboard. The digestion was cooled and carefully transferred into 100ml volumetric flask and made to mark with distilled water. Consequently, the green color obtained from distillation which was then titrated against 0.01N HCl contained in a burette. At the equivalent point, the green colour turned to violet/pink color which indicated that all nitrogen trapped an ammonium borate has been removed as ammonium chloride.

%N = Titre value x Atomic mass of Nitrogen x molarity of HCl. The total crude protein content was determined by multiplying percentage nitrogen by a constant factor of 6.26. %

Crude Protein value = %N x 6.26…..... (3.2)

3.2.3.2 Determination of petroleum ether extract

A gram of dried sample was weighed into the fat free extraction thimble and tightened with cotton wool. The thimble was placed in the extractor and fitted up with refluxed condenser with a 250ml soxhlet flask which has been previously dried in the oven, cooled in the dessicator and weighed. The soxhlet flask was then filled to full capacity with petroleum ether 40^{o}C – 60^{o}C boiling point. The soxhlet flask plus the condenser set is placed on the heating mantel adjusting to boiling temperature of the solvent for condensation of petroleum ether vapor. The solvent was left to siphon over several times for at least 10 - 12 times. Distillation was continued until the flask was practically dry. The flask which contained the fat or oil was detached, its exterior cleaned and dried to a constant weight in an oven.

Taken the initial weight of dry soxhlet flask as Wo and the final weight of oven dried flask + oil/fat as W_1

$$\% \text{ fat/oil} = \frac{W1 - Wo}{\text{Wt of sample}} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.3)$$

3.2.3.3 Determination of crude fiber

Two grams of the sample (W_1) was accurately weighed into the fiber flask and 100ml of 0.255N H_2SO_4 was added. The mixture was heated under reflux with the heating mantle. The hot mixture was filtered through a fiber sieve cloth. The filtrate obtained was thrown off and the residue was returned to the flask to which 100ml of 0.313M NaOH was added and heated under reflux for another one hour. The mixture was filtered through a fiber sieve cloth and 10ml of acetone was added to dissolve any organic constituent. The residue was washed with 50ml hot water twice on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven dried at 105^{o}C overnight to drive off moisture. The oven dried crucible containing the residue was cooled in desiccators and later weighed. For the determination of ash free, the fiber was put in a muffle furnace at 550^{o}C for four hours. The crucible containing white and grey ash (free of carbonaceous material) was cooled in desiccators and weighed to obtain W_2. The difference $W_1 - W_2$ gave the weight of ash free fiber.

$$\% \text{ Fiber} = \frac{W1 - W2}{\text{Wt of sample}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.4)$$

Ash content

Two grams of the sample was weighed in porcelain crucible; this was transferred into the muffled furnace at 550 degree Celsius and left for about four hours. About this time it had turned to white ash. The crucible and its content were cooled to about 100ºC in air then to room temperature in a dessicator and weighed. This was done in duplicate.

$$\% \text{ Ash content} = \frac{\text{weight of ash}}{\text{Wt of sample}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.5)$$

Moisture content

Two grams of the sample was weighed into a previously dried and weighed crucible. The crucible and sample taken were then transferred into the oven set at 100ºC index and allowed to dry overnight. At the end of the 24 hours the crucible plus sample were removed from the oven and transferred to the desiccators and cooled for 10 minutes and weighed.

where: The weight of empty crucible = W_o

Weight of crucible + sample = W_1

Weight of crucible + oven dried sample = W_3

$$\% \text{ moisture content} = \frac{W_3 - W_o}{W_1 - W_o} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.6)$$

3.2.3.4 Determination of mineral elements

Weight one gram of each of the triplicate dried samples in a crucible was ashed at 550°C in a muffle furnace for 4 hr. The ash was later dissolved in 100 mL volumetric flask with de-ionized water and 10 mL of concentrated hydrochloric acid was added and filtered. The filtrate was made up to 50 mL with 0.1 M HCl. Calcium (Ca), manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu) were determined.

$$\text{mg/100g of metal content} = \frac{[(C_s - C_b) \times V]}{[w]} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.7)$$

Where, C_s = Concentration of Sample in ppm

C_b= Concentration of blank in ppm

V= Volume (ml) of extract

W= weight (g) of samples

3.2.4 Response variable and factors selection of the process

The response variable for this experiment was the yield of the extract crude oil extracted from the root of ginger.. There were many factors which affects the extraction of the extract crude ginger oil from the root of the rhizome *zingiber officinale* plant. These factors are residence time, the ratio of ginger root powder with solvent extraction and particle size. These three important parameters had different levels. Particle size was investigated in three levels 1 µm, 3 µm and 5µm. Residence time also considered in three levels that was one hour, two hours, and three hours. The third independent variable which was ratio of ginger root powder to solvent (n-hexane) was investigated in three levels 1:8, 1: 10 and 1: 12 ratio.

3.2.5. Experimental design and Statistical analysis

As it was mentioned above the variable of interest in this research are particle size, residence time and ratio of the ginger root powder to solvent. The variables also considered in different levels and their interaction effects on the yield of the extract crude ginger oil. To investigate those variables, factorial design is advisable and the most efficient. By the factorial design, in each complete trial or replication of the experiment all possible combination of the levels of the factors is investigated. In this experiment the levels of the factors are three levels as a result Response Surface Methodology (RSM) with Central Composite Design (CCD) with three levels factorial was applied and thirty two experiments with replications were studied. A Central Composite Design (CCD) with three levels factorial gave a total of 32 experimental runs.

Table 3.1 Different level of factors associated with the experiment.

Variables	**Unit**	**Levels**		
		-1	**0**	**+1**
Solid to solvent ratio	g/ml	1:8	1:10	1:12
Extraction time	hr	1	2	3
Particle size	µm	1	3	5

The statistical software package Design Expert version 7.0.0 (Stat Ease Inc., Minneapolis, USA) was used to generate the experimental data and develops the regression model. The statistical significance determined by ANOVA and determines the optimum Condition.

3.2.6 Effect of extract crude ginger oil on microbial

The microorganisms selected for the study are *Bacillus subtilis* (gram positive bacteria), *Pseudomonas aeruginosa* (gram negative), three fungi species namely *Candida albicans*, *Aspergillus niger*, *Penicillium spp*, *Saccharomyces cerevisiae* (yeast) and one dermatophyte which is a Trichoderma spp.

Experimental procedure of Antimicrobial activity

A standard disc diffusion method by Baurer et al (2007) was used. In each experiment, microorganisms were cultured at 37^{o}c for 4 hr and prepared to turbidity equivalent to 0.5 McFarland standards (National Committee of Clinical Laboratory Standards, 2000).

Then 100 µL of the suspension was spread on the test plate (Nutrient Agar). Sterile discs (6 mm diameter) were impregnated with 10 µL of the extract crude ginger oil and placed on the surface of the test plate. Control discs were saturated with tetracycline (10 µg/disc).Plates was subsequently incubated at the appropriate temp for 24 hrs and zones of inhibition were calculated by measuring the diameter in mm. In the case of fungi, dermatophyte and yeast, the test was performed in sterile Petri dishes containing saboraud dextrose agar. The oils were adsorbed on sterile paper disc and placed on the surface of the medium previously inoculated with a suspension of bacteria, fungus, yeast and dermatophyte. All Petri dishes were sealed with a sterile paraffin film to avoid evaporation of the test samples and incubated at 28^{0}C.The zone of inhibition was determined by measuring the diameter of the clear zone around each disc. The standard antibiotics griseofulvin was used for dermatophyte and Nyastatin used for fungi. The test was conducted in Microbiology Laboratory of the EFMHACA.

3.2.7 Identification of Chemical Components of Ginger (*Zingiber Officinale* Roscoe)

The extracted crude oil samples were analyzed using Gas Chromatography Mass Spectrometry (GC - MS) Agilent 6890 gas chromatography instrument coupled to Agilent 5973 mass spectrometer and Agilent Chem software in order to identify their chemical constituents. This is an essential method to evaluate the quality of the oil samples, the operating parameters of GC-MS was as followed: system operating in EI mode (70 eV), equipped with a split/splitness injector (280ºC, split ratio 1:20), using DB -5 column (30 x 0.2 5 mm i.d x 0. 2 5 mm). The temperature program is 50ºC (5 min) rising to 300ºC at rate of 5ºC/min. Injector and detector temperature was 280ºC .Helium was used as carrier gas at a flow rate 1 mL /min.

CHAPTER FOUR

4.0 Result and Discussion

In this section output of the results obtained from solvent extraction of ginger extract crude oil, Characterization and preservative effect of the extracted oil were discussed. The effects of main and interaction factors and the optimum levels of the parameters were also investigated. The model of the output data was also developed the adequacy of it was investigated.

4.1 Characterization of the extract crude oil from ginger root

Proximate composition of the powdered ginger root sample is present in Table 4.1. This shows that crude protein is 11.52%; this result indicates that ginger is a good source of protein. The crude fiber content was 4.02%.The crude fiber of the sample was moderate and it reduces serum cholesterol levels.

The petroleum ether extract content of the sample was 3.87% and the crude fat content was moderate because samples low in fat is advantageous as they may reduce the risk of coronary heart disease and lower the risk of hypertension. The ash content of the sample was 7.64%; the ash content indicates the level of essential or non-essential mineral elements in the sample.

The mineral elemental composition of ginger root samples as shown in Table 4.2 indicates that ginger is rich in essential minerals like: calcium 85.00 mg/100g, Iron 48.96 mg/100g, Zinc 2.33 mg/100g, Manganese 5.90 mg/100g and Copper 0.75 mg/100g. The result on mineral composition is in agreement with some medicinal plant species which shows calcium as the pre-dominant element. It was reported that the dietary intake of essential minerals should be > 50 mg/day. The essential mineral like calcium is important in extra- cellular and intra-cellular body functions and as components responsible for the building block of structural component in human body. Minerals like Iron, even if present in threshold level can act as anti-oxidant and are involved in strengthening the immune system.

Whereas, Zinc are known to prevents cardiomyopathy, muscle degeneration, growth retardation and bleeding disorder. Therefore, the presence of these minerals in ginger root provides bases for their use in food application.

Table 4.1 Proximate composition of ginger root

Parameters	Amount (%)
Crude Protein	11.52
Crude fat	4.02
Ether extract	3.87
Ash content	7.64
Moisture content	9.75

Table 4.2 Mineral composition of ginger root

Parameters	Amount in mg/100
Calcium	85.00
Copper	0.75
Zinc	2.33
Iron	48.96
Manganese	5.90

All experiments were done in replicate and averaged

4.2 Effect of extract crude ginger oil on microbials

Table 4.3 Diameter bacterial growth inhibition after 24 hours

Strains	Diameter of bacterial growth inhibition(mm)		Reference compound
	Fresh ginger extract oil	Dry ginger extract oil	
Bacillus subtilis	6.02 ±0.04	5.3 ± 0.05	7.20 ± 0.06
Pseudomonas aeruginosa	7.2 ± 0.06	9.04± 0.11	8.10 ± 0.08
Aspergillus niger	8.22 ± 0.03	5.5 ± 0.04	8.25 ± 0.03

Pencillium spp	-	4.2 ± 0.11	8.08 ± 0.12
Candida albicans	12.13 ± 0.02	12.22 ± 0.3	12.09± 011
Saccharomyces cerevisiae	6.05 ± 0.06	7.1 ± 0.04	12.14 ± 0.04
Trichoderma	-	-	12.09± 011
E.coli	6.97 ± 0.6	7.02 ± 0.03	7.20 ± 0.06
S. aureus	4.0 ± 0.80	5.45 ± 0.04	8.10 ± 0.08

The values reported in the above table are the average of duplicates followed by standard deviations

Reference compounds: Tetracycline for bacteria, Nyastatin for fungi and Griseofulvin for dermatophyte.

From the Table 4.3 we can conclude the preservative effect of extracted ginger oil is equivalent of the synthetic preservative compounds. So that the ginger extract oil can substitute the synthetic preservative.

4.3. Effect of extraction parameters on yield (response)

In this study the effect of extraction parameters (solvent amount, particle size and extraction/contact time) on the yield of extract crude ginger oil were studied. Table 4.4 shows the effect of extraction parameters on crude ginger oil yield. Yield of crude extract ginger oil was determined on the basis of input-output results of statistical design. Based on the result analysis, it has significant ($p < 0.05$) positive effect on extract crude ginger oil yield. The highest (26.24 %) and the lowest (14.36 %) yield of extracts were obtained at 160 ml and 240 ml, respectively. The highest yield extracts was obtained at 160ml solvent, a particle size of 3 μm and contact time of 3 hours. Design expert software shows the effect of extraction parameters on yield of ginger oil in Table 4.4.

Table 4.4 CCD experimental designs showing the effect of extraction parameters on yield of extracts crude ginger oil.

Run	Etraction Parameters			Ginger oil yield(%) (ml)
	Factor: 1 A:solvent Ratio (ml)	Factor:2 B:Particlesize (μm)	Factor:3 C:Extraction time(hr)	
1	1:12	3	3	18.8796
2	1:8	3	3	26.1246
3	1:10	3	2	24.2568
4	1:10	3	2	23.8974
5	1:10	3	1	23.7698
6	1:10	3	2	23.6259
7	1:8	5	1	25.7896
8	1:10	3	2	20.1266
9	1:10	3	2	21.9875
10	1:10	3	3	22.1235
11	1:8	1	3	25.2357
12	1:12	1	3	15.5689
13	1:12	3	2	17.4875
14	1:8	3	2	25.9865
15	1:12	1	1	14.3569
16	1:8	3	1	25.7856
17	1:10	5	2	22.6895
18	1:12	5	1	18.4689
19	1:12	5	3	17.5236
20	1:10	1	1	22.5689
21	1:8	5	3	25.9638
22	1:10	5	1	21.8796
23	1:8	3	3	26.2356
24	1:8	5	2	25.6897
25	1:8	1	1	24.5689
26	1:10	3	2	22.3568
27	1:12	1	2	16.8654
28	1:10	1	2	20.1246
29	1:12	5	2	17.3569
30	1:10	5	3	22.3589
31	1:12	3	1	17.3458
32	1:10	1	3	22.4568

All experiments were done in duplicate and the result were averaged

Fig. 4.1 represents the parameter of particle size increases at the reference point and the yield also increases. After reference point slightly decreases, the production of ginger oil yield also decreases. When the size is big, there is longer diffusion or mass transfer between solvent and the plant particles. The parameter of solvent ration decreases as the amount of solvent increases and the ginger oil yield decreases the high amount of solvent used will be reduced the inter solubility of components and the yield also reduced. The parameter of extraction time is insignificant on the production of ginger extract crude oil.

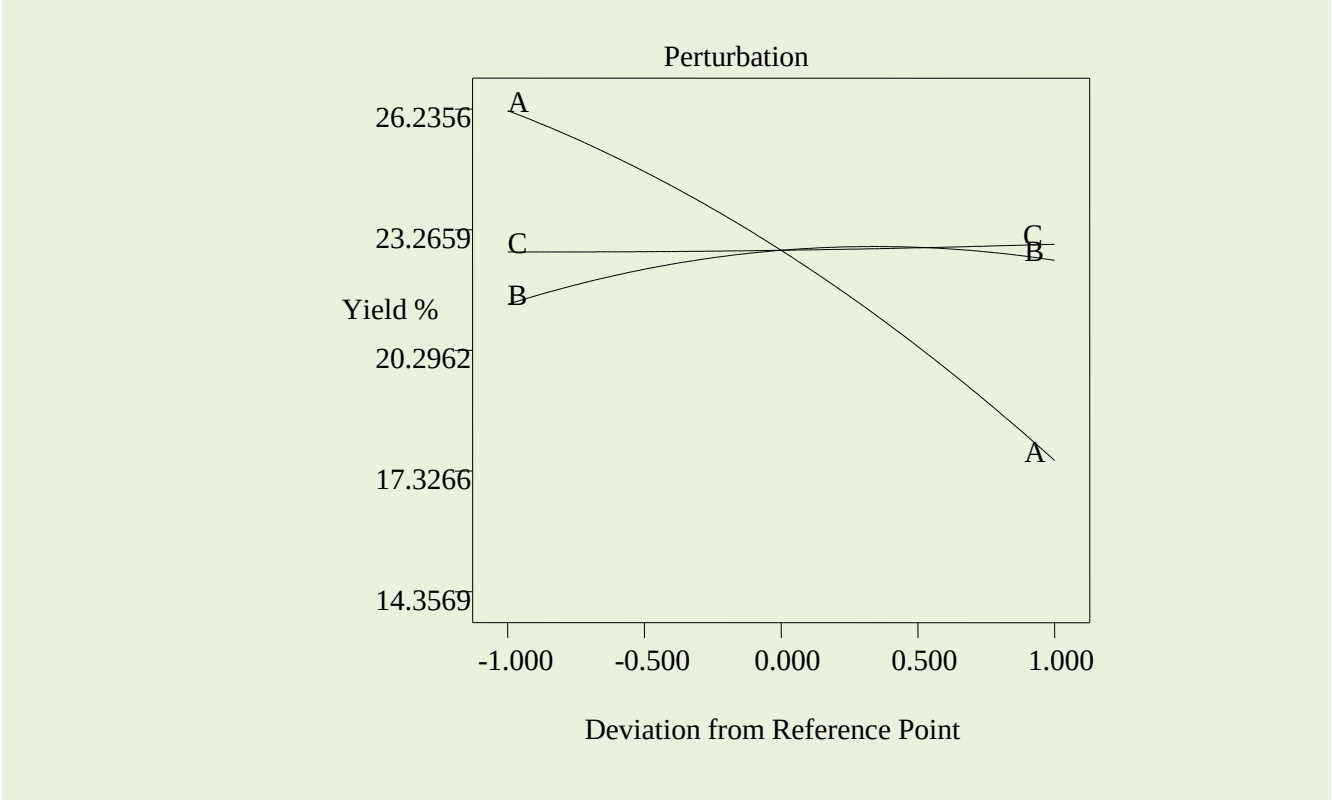


Figure 4.1 Perturbation graphs showing the interaction factors (A: solvent ratio, B: Particle size and C: Extraction time).

4.3.1. Validation of the experimental model for yield of ginger crude oil extracts

Table 4.4 summarizes the result obtained with the experimental design which was aimed in determining the conditions that favors maximum yield increase in crude ginger oil extracts. A quadratic model and also the linear model shows the fitness of the data model for predicting response; yield of crude ginger oil extracts was given in equation (4.1) for quadratic and equation (4.2) for linear.

Yield (Y) = +22.76 - 4.3 * A + 0.54 * B +0.095 * C - 0.87 * A^2 - 0.79 * B^2 + 0.055 * C^2 +

0.43 * A * B +0.05 * A * C -0.17 * B * C …… 4.1

Yield (Y) = +21.86 - 4.31 * A + 0.54 * B + 0.095 * C ……..4.2

Where A is extraction solvent volume, B is particle size and C is extraction/contact time, whereas the number 22.76 is the intercept term. These two model gave the same low probability $P_{model} > F$= 0.0001 and $P_{model} > F$ =0.0001 when tested by Analysis of variance (ANOVA).The analysis of variance is given in Table 4.5 for quadratic and Table 4.6 for linear.

Table 4.5 ANOVA for Response Surface Quadratic model

Source Variation	Sum of Square	DF	Mean Squares	F- Value	Probability > F
Model	355.03	9	39.45	34.70	<0.0001 significant
A:solvent Ratio	333.91	1	333.91	293.68	<0.0001
B:Particle size	5.27	1	5.27	4.63	0.0426
C:Extraction time	0.16	1	0.16	0.14	0.7105
A^2	5.39	1	5.39	4.74	0.0405
B^2	4.46	1	4.46	3.92	0.0603
C^2	0.022	1	0.022	0.019	0.8916
AB	2.21	1	2.21	1.95	0.1767
AC	0.032	1	0.032	0.028	0.8681
BC	0.35	1	0.35	0.31	0.5830
Residual	25.01	22	1.14		
Lack of Fit	13.05	17	0.77	0.32	0.9645not significant
Pure error	11.96	5	2.39		
Cor. Total	380.04	31			

The Model F-value of 34.70 implies the model is significant.There is only a 0.01% chance that a model F-value is large could occur due to noise. Values of Prob > F less than 0.0500 indicate model terms are significant. In this case A, B, A^2 are significant model terms.Values greater than 0.05 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model.The Lack of Fit F-value of 0.32 implies the Lack of Fit is not significant relative to the pure error. There is a 96.45% chance that a Lack of Fit F-value this large could occur due to noise.

Table 4.6 ANOVA for Response Surface Linear model

Source Variation	Sum of Square	DF	Mean Squares	F - Value	Probability > F
Model	339.34	3	113.11	77.81	<0.0001 significant
A:solvent Ratio	333.91	1	333.91	229.70	<0.0001
B:Particle size	5.27	1	5.27	3.62	0.0673
C:Extraction time	0.16	1	0.16	0.11	0.7419
Residual	40.70	28	1.45		
Lack of Fit	28.74	23	1.25	0.52	0.8692 not significant
Pure error	11.96	5	2.39		
Cor Total	380.04	31			

The Model F-value of 77.81 implies the model is significant. There is only a 0.01% chance that a model F-value is large could occur due to noise.Values of Prob > F less than 0.05 indicate model terms are significant. In this case A is significant model terms. Values greater than 0.05 indicate the model terms are not significant. If there are many insignificant model terms, the model reduction may improve your model.The Lack of Fit F-value of 0.52 implies the Lack of Fit is not significant relative to the pure error. There is a 86.92% chance that a Lack of Fit F-value is large could occur due to noise. Non-significant lack of fit is good , we want the model to fit.

The goodness of fit was evaluated by the coefficients of determination (R^2), which was 0.9342 and 0.8929 and this reveals that 93.42 % and 89.29 %, of the data was explained by the two selected models. The adequate precision of 18.009 and 23.190 for quadratic and linear model respectively for extract crude ginger oil were greater than 4, which indicates the two models could be used to investigate the design space.

4.3.2 Model Adequacy Check

Before the model implemented for different applications it should satisfy different criteria such as the normal distribution of the residuals. Unless the model should not satisfy these criteria it is not advisable to use the model for different purpose rather other methods are applied to adjust the model in desirable way.

Normal probability plot of the residuals

The normal probability plot of the residuals showed that, it follows almost a straight line which implies residuals are approximately normally distributed that satisfy the most important assumption in any model. Normal probability plots of residuals shown Fig.4.2 and Plot of residuals Vs predicted values shown Fig.4.3

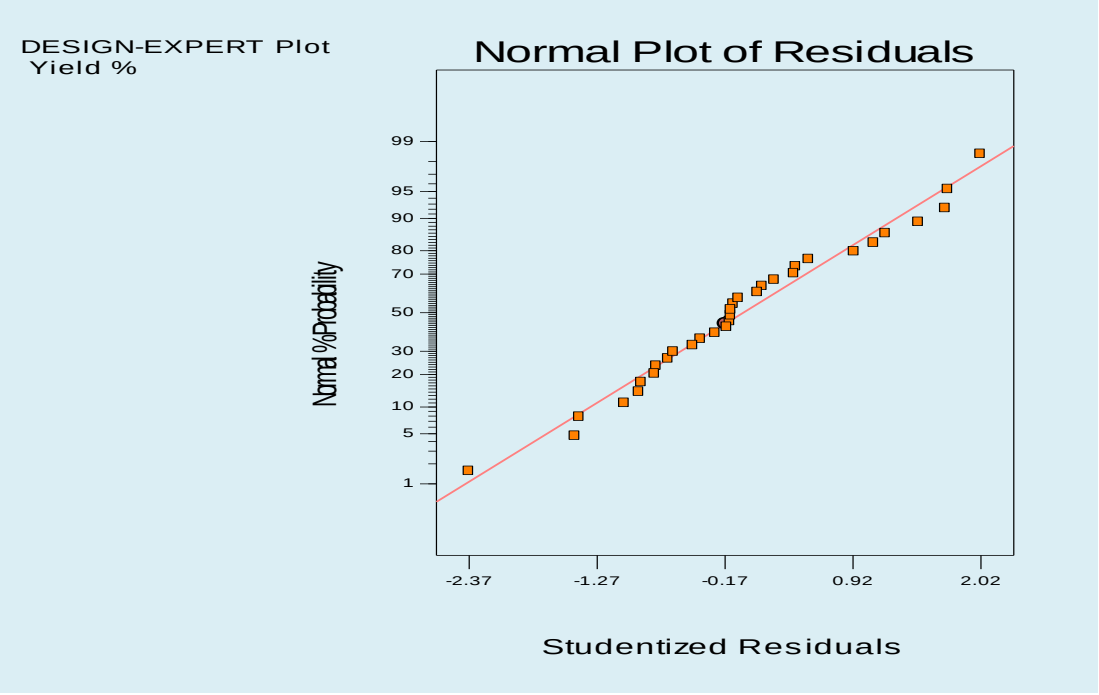


Figure 4.2 Normal probability plots of residuals

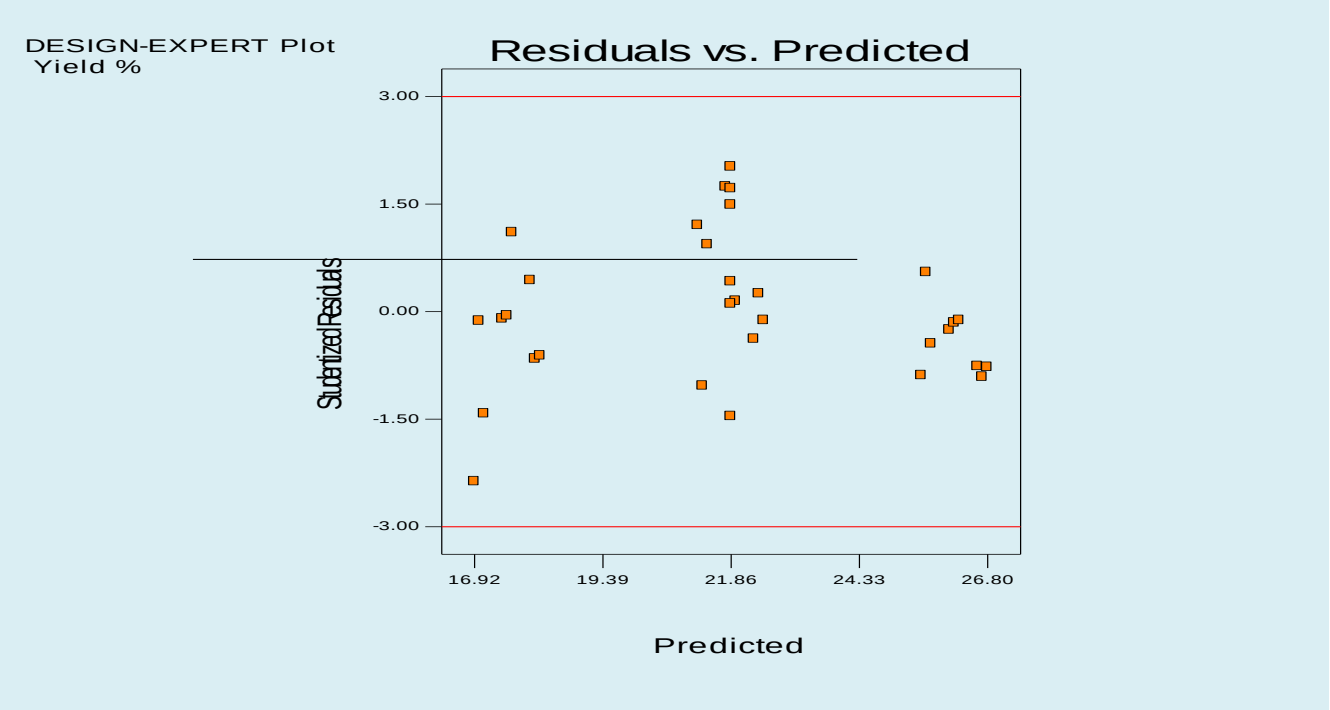


Figure 4.3 Plot of residuals Vs predicted values

Fig.4.3 shows that the plot of residuals verses predicted value did not follow any pattern, it is random it implies that the model is adequate.

4.4 Chemical Composition Analysis of Ginger Oil

Table 4.7 presents the respective retention time and concentrations of compounds present in the extracted oil. Figure 2 shows the chemical structure of some compounds identified by GC-MS.As indicated in Table 4.7 zingiberene is the predominant compound belonging to the sesquiterpene hydrocarbons and constituted approximately 30 % of the total extracted essential oil. The findings are in agreement with previous work carried out by Sultan *et al*. (2005). Totally seventeen compounds are identified in the essential oil examined. The chemical constituents of the essential oils extracted as mentioned in Table 4.6 belong to sesquiterpene hydrocarbons namely zingiberene, AR-curcumene, ß-sesquiphellandrene while the oxygenated monoterpene namely Endo- borneol and geraniol are present at lower concentrations and have more contributions to the flavouring characteristics of the oil. The monoterpene hydrocarbons, camphene and ß -phellandrene and sesquiterpene alcohols, nerolidol and α-eudesmol are also present in the extracted oil. The main constituent of the extract oil is Zingiberene (29.74 %) which is the major sesquiterpene hydrocarbon of ginger essential oils and this compound was identified and isolated. Zingiberene has biological activities such as ant-fever, antivirus and ant-gestation (Millar & Notprod, 1998).

Table 4.7 Chemical composition and concentrations of compounds present in ginger oil

Compound	Concentration (%)	Retention time (RT)
Camphene	0.72	9.12
ß - Phellandrene	0.92	11.15
Endo -Borneol	0.97	14.78
Genariol	0.98	17.05
Geraly Acetate	1.36	19.55
AR -Curcumene	15.76	22.01
Zingiberene	29.74	22.25
α- Farnesene	5.61	22.34
ß - sesquiphellandrene	10.21	22.56
4,5-Dimethayl-11-Methylene Tricycle	2.45	22.46
γ - Cadinene	3.46	22.71
ß - Sequiphellandrene	15.47	22.90
δ - Cadinene	0.65	23.01
Nerolidol	2.00	23.56
7-Aipha –(1-hydroxyl Methylethyl)	1.98	25.25
Germacrene	1.10	25.40
α- Eudesmol	3.23	36.02

All experiments were done in triplicate and the result were averaged

4.5 Optimization of extracted crude ginger oil yield

The objective of the study was to obtain maximum yield in the given interval of the investigated independent variables. Table 4.4 shows the actual factorial experiment and response value that were designed by Design Experiment and were performed which comprised three factors predictors (solvent ratio, extraction time and particle size) and one response criterion (ginger oil yield).Result for Analysis of Variance (ANOVA) is shown in figure 4.5.The significant level of each coefficient is determined by p value <0.0001. From ANOVA, the model is statistically significant with F-value of 34.7, thus the model is accepted and there are only 0.01% chances that this due to noise. In this model, A (solvent ratio), B (Particle size), B (Extraction Time), A, B and A^2 (Solvent ratio) are significant model terms with significant p-value <0.0001. These factors have significant effect on response individually or via interaction.

The optimum ginger oil yield at solvent ratio 160 ml to 20 g, extraction time is 3hr and using 3 μm particle sizes and the desirability parameter was selected to optimize the process.

Upon optimization, a solution combining three factors above suggest at desirability value of one, to obtained maximum ginger oil yield of 26.24%. A confirmation run to validate the model were done at a selected optimal parameters and revealed results of average ginger oil yield of 26.24% (Fig. 4.3).The following data took from design expert output and among many possible combinations; ten of the highest yield offering combination would take. Possible combination of the treatments shown in Table 4.8.

Table 4.8 Ten highest yields offering possible combination of the treatments in report form

Solutions no.	Solvent ratio	Particle size	Extraction time	Yield (%)	Desirability
1	8.01	3.10	3.00	26.283	1.000
2	8.02	2.98	2.89	26.2545	1.000
3	8.00	3.11	2.60	26.2382	1.000
4	8.03	2.62	2.98	26.2388	1.000
5	8.01	2.70	2.74	26.2387	1.000
6	8.00	3.42	1.00	26.2354	1.000

7	8.00	3.05	2.42	26.2269	0.999
8	8.00	3.30	1.10	26.2264	0.999
9	8.00	3.33	1.11	26.2262	0.999
10	8.00	3.69	3.00	26.1821	0.995

The desirability greater than 70 % combination of factors was advisable. As a result in Table 4.8 the all possible combinations of the factors gave maximum yield.

The highest yield offering possible combination of the treatments in ramps form for the top two possible combinations agree with the results of the above table expression. The following Fig. 4.4 and Fig. 4.5 show the first and the second high yield offering combination of the treatments.

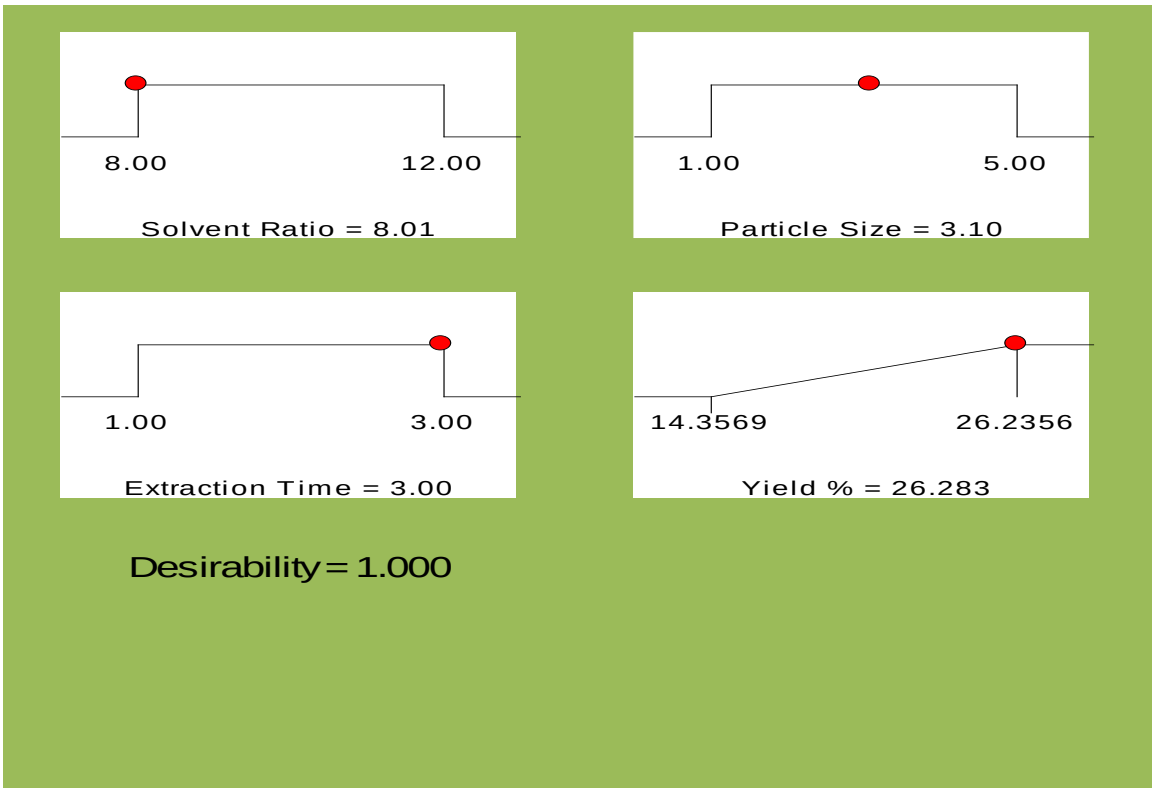


Fig. 4.4 The highest yield offering combination of the treatments

The second highest yield obtained at the combination of treatments of solvent ratio 8.02 with the residence time of 2.89 hours and 2.98 µm for particle size.

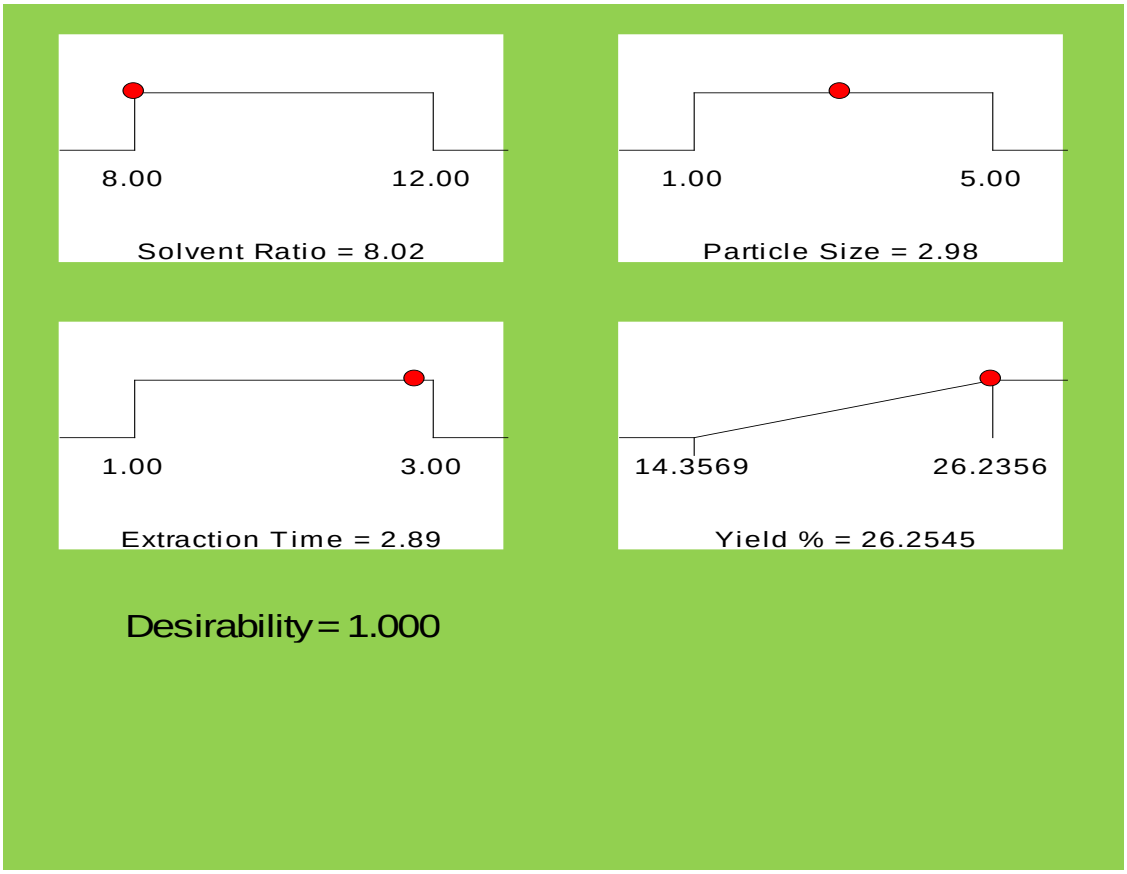


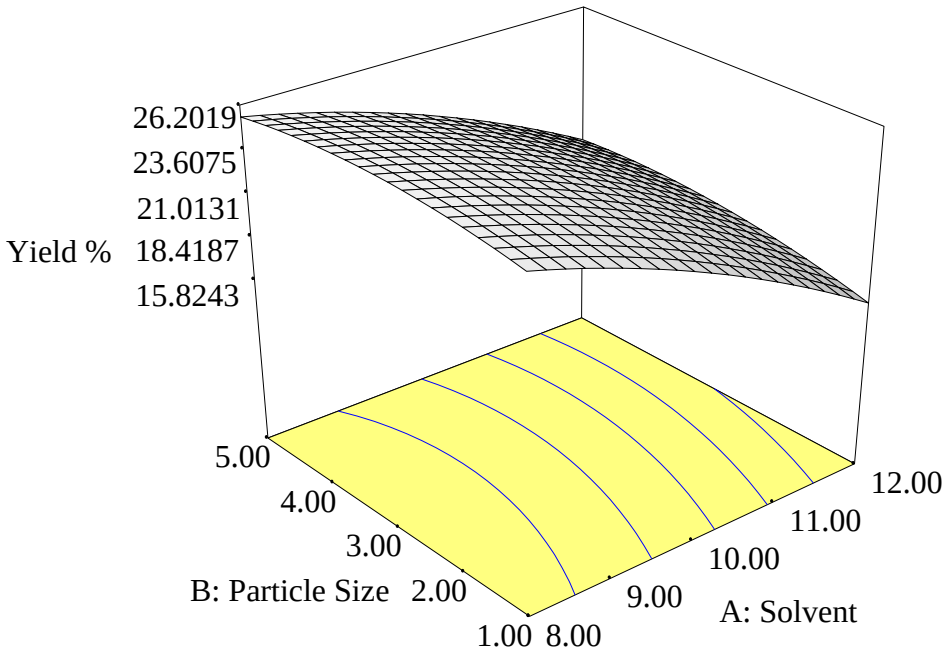
Fig. 4.5 The second highest possible combination of treatments

The relationship between independent and dependent variables was graphically represented by 3D response surface plots generated by the model is shown in the Fig. 4.6. Different shapes of the contour plots indicate different interactions between the variables. Individually, as solvent ratio increases the ginger oil production will drop and further increase in ratio will not be beneficial. This may be due to oil cells that are not rebuilt due to fully rupture of parenchyma cell wall.

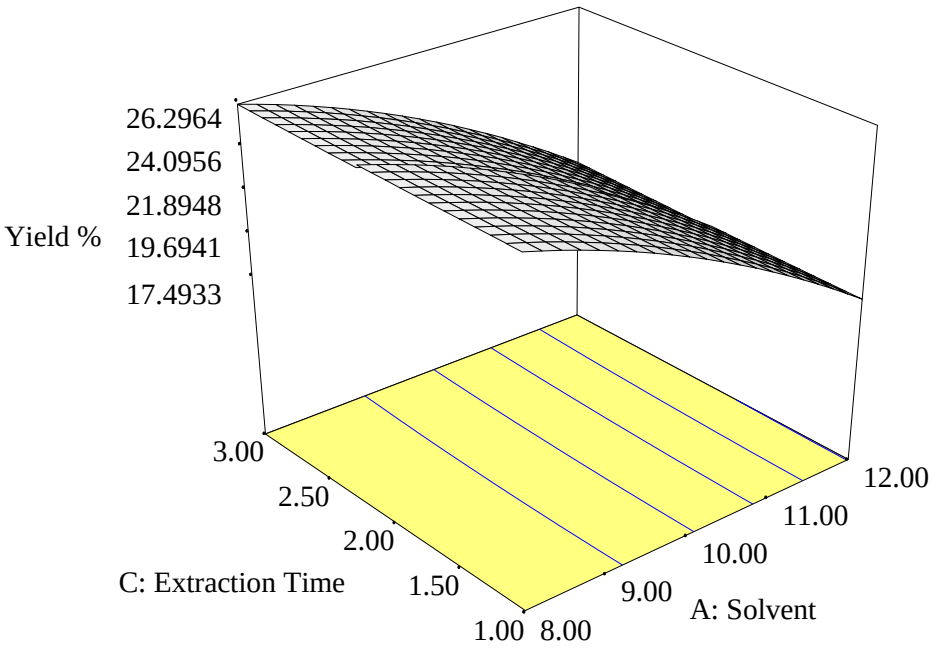
The ginger oil production is increase as particle size is at certain point. After certain point the ginger production of oil will drop and further increase in particle size will not be useful to get the maximum yield of crude ginger oil. When the size is big, there is longer diffusion or mass transfer between solvent and the plant particles. Thus the interaction between solvent ratio and particle size is important as stated in these studies to optimized ginger oil yield.

Increase of extraction time from 1hr to 3 hr and at the lowest volume of solvent improves the extract ginger oil yield. Further increasing extraction time may accelerate chemical condensation and possibly rejected as residue of bioactive compound in extraction process, which resulted in the lower extraction yield. Fig. 4.6 (a) describes the effect of solvent extraction ratio (A) and particle size (B) on the yield of crude ginger oil extracts, (b) Response surface method plot of extraction time (C) and solvent

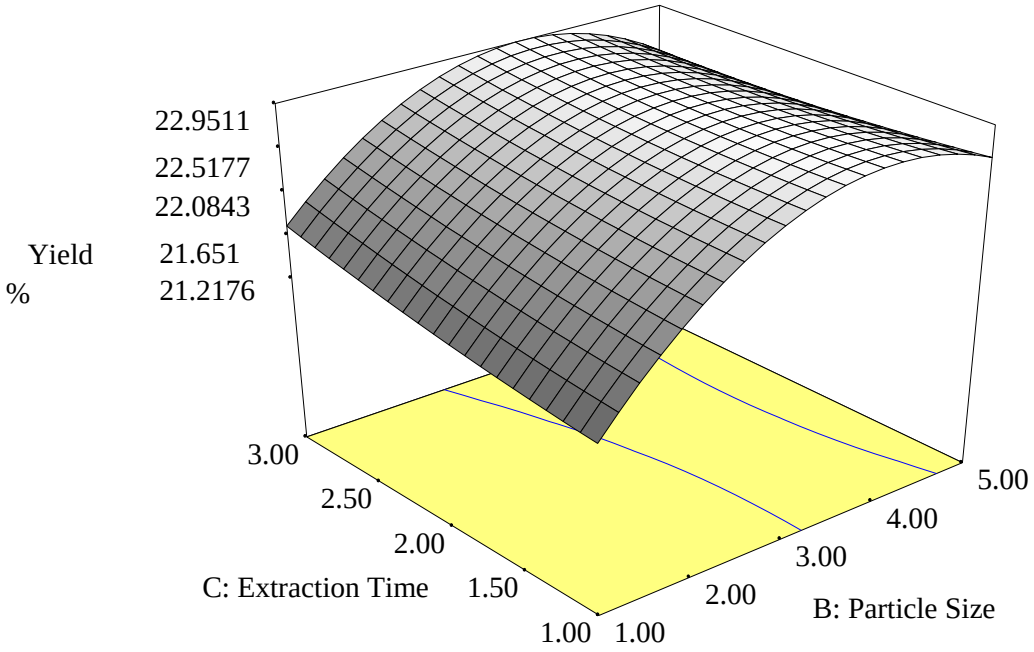
concentration and (c) Response surface method plot of extraction time and particle size on ginger oil yield**.** 3D response surface method plot showing the effect of extraction parameters on yield of extract ginger oil in Fig. 4.6 (a), (b) and (c).



(a) Response surface method plot of concentration of solvent and the particle size



(b) Response surface method plot of extraction time and solvent concentration



(c) Response surface method plot of extraction time and particle size

Fig. 4.6 (a), (b), (c) 3D response surface method plot showing the effect of extraction parameters on yield of extract ginger oil.

CHAPTER FIVE

5.0 Conclusion and Recommendation

5.1 Conclusion

This study indicates that different factors had different effects on the yield of extract ginger oil product. Response Surface Methodology (RSM) with Central Composite Design (CCD) with three levels factorial design is the statistical analysis used for optimization process of ginger oil yield. Extraction of extract ginger oil was conducted by varying extraction parameters, namely solvent concentration ratio, particle size and contact time. Extraction was conducted at solvent amount (160, 200 and 240 ml), particle size (1 μm, 3 μm and 5 μm) and contact time (1hr, 2hr and 3hr). From the optimized value, to obtained maximum ginger oil yield of 26.23%, the optimal solvent ratio to sample value is 160 ml to 20 g (1:8 solid - to - solvent), 3 μm of particle size and the contact time is 3 hours by using n- hexane extraction solvent.

A confirmation run have validate the model is accurate. In the model adequacy checking, the normal probability plot of the residuals showed a straight line which implies that the residuals were normally distributed.

In conclusion, this study can be in food and medicinal sectors which have interest to engage in manufacturing natural antioxidant from ginger oil and also the results indicate it can substitutes the import synthetic preservative and save hard currency for the country.

5.2 Recommendation

Ethiopia should encourage the production of this valuable plant and value adds products of it for multi-directional purpose. The benefit of the products extends to health and socio-economic advantage. The product of natural organic ginger has many benefits regarding to solve the health problem and also the result shows the organic products can substitute the synthetic product which imports from abroad country.

Different governmental or private sectors that are interested in agro industry area recommend participating in this profitable area. If this effort should be done, it would have significant positive effect on the diversification of the Gross Domestic Product (GDP) of Ethiopia as well as the poverty and unemployment reduction. Researchers should investigate further on the production of ginger in our country and the benefits obtained from the product have to analyze deeply to encourage the pharmaceutical industries and other investors locally.

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Appendices

Appendix A: The design layout of the experiment designed by design expert software.

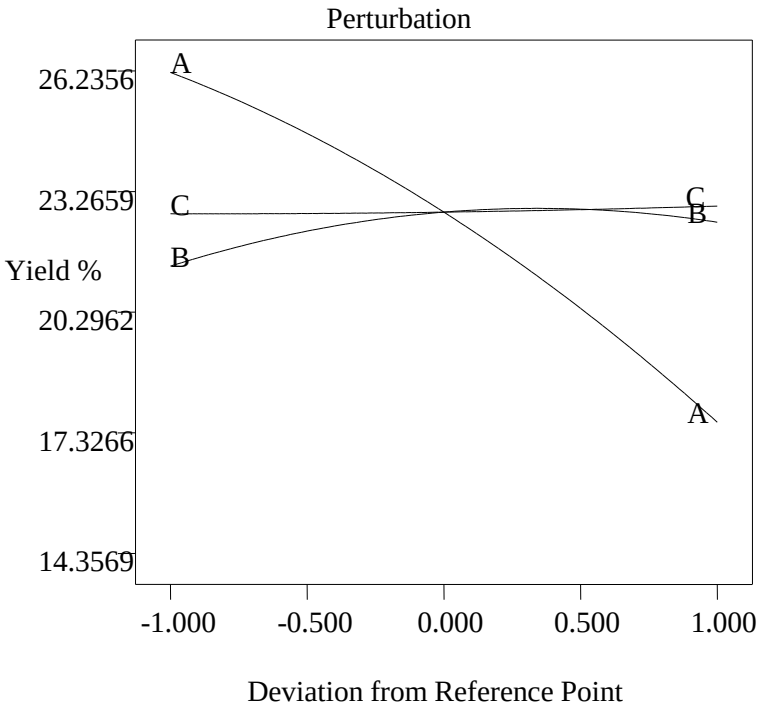
Run	Etraction Parameters			Ginger oil yield(%) (ml)
	Factor: 1 A:solvent Ratio (ml)	Factor:2 B:Particlesize (μm)	Factor:3 C:Extraction time(hr)	
1	1:12	3	3	18.8796
2	1:8	3	3	26.1246
3	1:10	3	2	24.2568
4	1:10	3	2	23.8974
5	1:10	3	1	23.7698
6	1:10	3	2	23.6259
7	1:8	5	1	25.7896
8	1:10	3	2	20.1266
9	1:10	3	2	21.9875
10	1:10	3	3	22.1235
11	1:8	1	3	25.2357
12	1:12	1	3	15.5689
13	1:12	3	2	17.4875
14	1:8	3	2	25.9865
15	1:12	1	1	14.3569
16	1:8	3	1	25.7856
17	1:10	5	2	22.6895
18	1:12	5	1	18.4689
19	1:12	5	3	17.5236
20	1:10	1	1	22.5689
21	1:8	5	3	25.9638
22	1:10	5	1	21.8796
23	1:8	3	3	26.2356
24	1:8	5	2	25.6897
25	1:8	1	1	24.5689
26	1:10	3	2	22.3568
27	1:12	1	2	16.8654
28	1:10	1	2	20.1246
29	1:12	5	2	17.3569
30	1:10	5	3	22.3589
31	1:12	3	1	17.3458
32	1:10	1	3	22.4568

Appendix B: Diameter bacterial growth inhibition after 24 hours

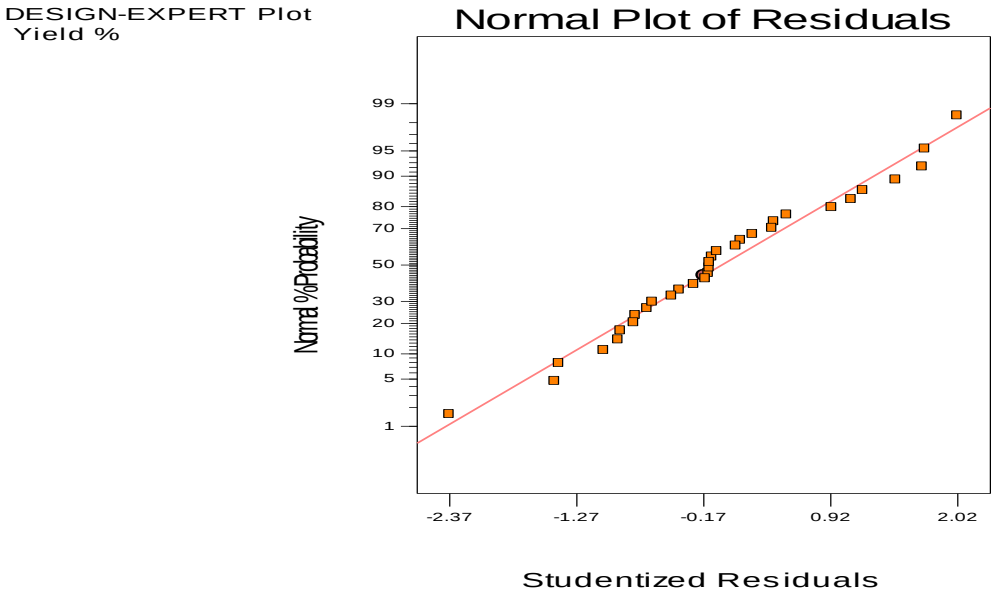
Strains	Diameter of bacterial growth inhibition(mm)		Reference compound
	Fresh ginger extract oil	Dry ginger extract oil	
Bacillus subtilis	6.02 ±0.04	5.3 ± 0.05	7.20 ± 0.06
Pseudomonas aeruginosa	7.2 ± 0.06	9.04± 0.11	8.10 ± 0.08
Aspergillus niger	8.22 ± 0.03	5.5 ± 0.04	8.25 ± 0.03
Pencillium spp	-	4.2 ± 0.11	8.08 ± 0.12
Candida albicans	12.13 ± 0.02	12.22 ± 0.3	12.09± 011
Saccharomyces cerevisiae	6.05 ± 0.06	7.1 ± 0.04	12.14 ± 0.04
Trichoderma	-	-	12.09± 011
E.coli	6.97 ± 0.6	7.02 ± 0.03	7.20 ± 0.06
S. aureus	4.0 ± 0.80	5.45 ± 0.04	8.10 ± 0.08

Appendix: C sox let apparatus and ginger extraction shooted photo during the experimental Session

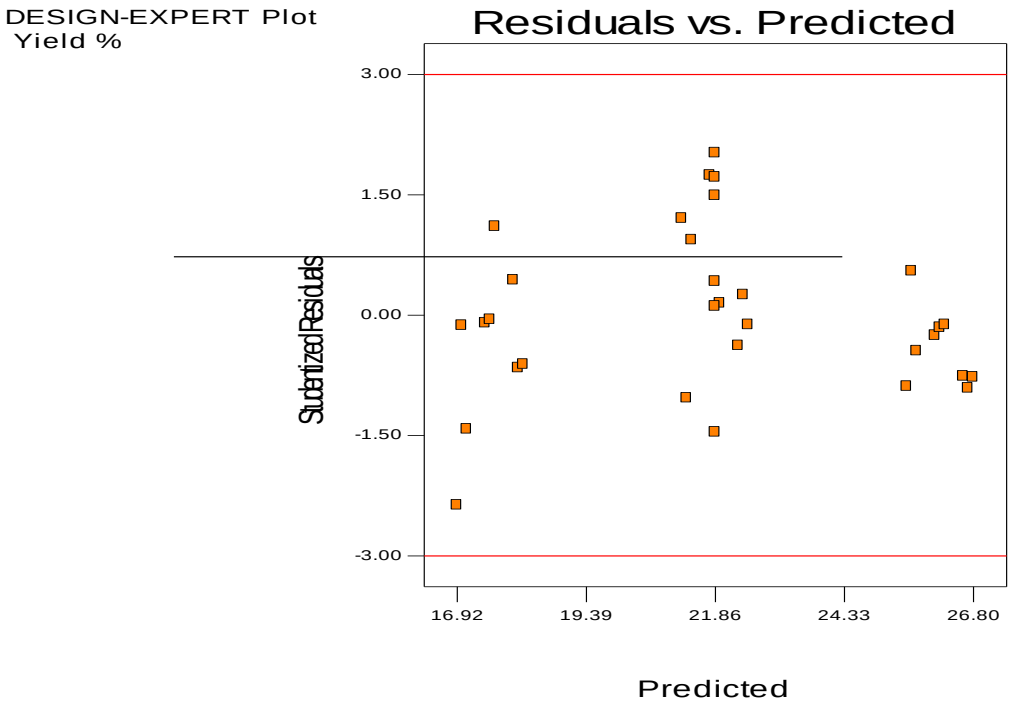
Appendix D: Perturbation graphs showing the interaction factors



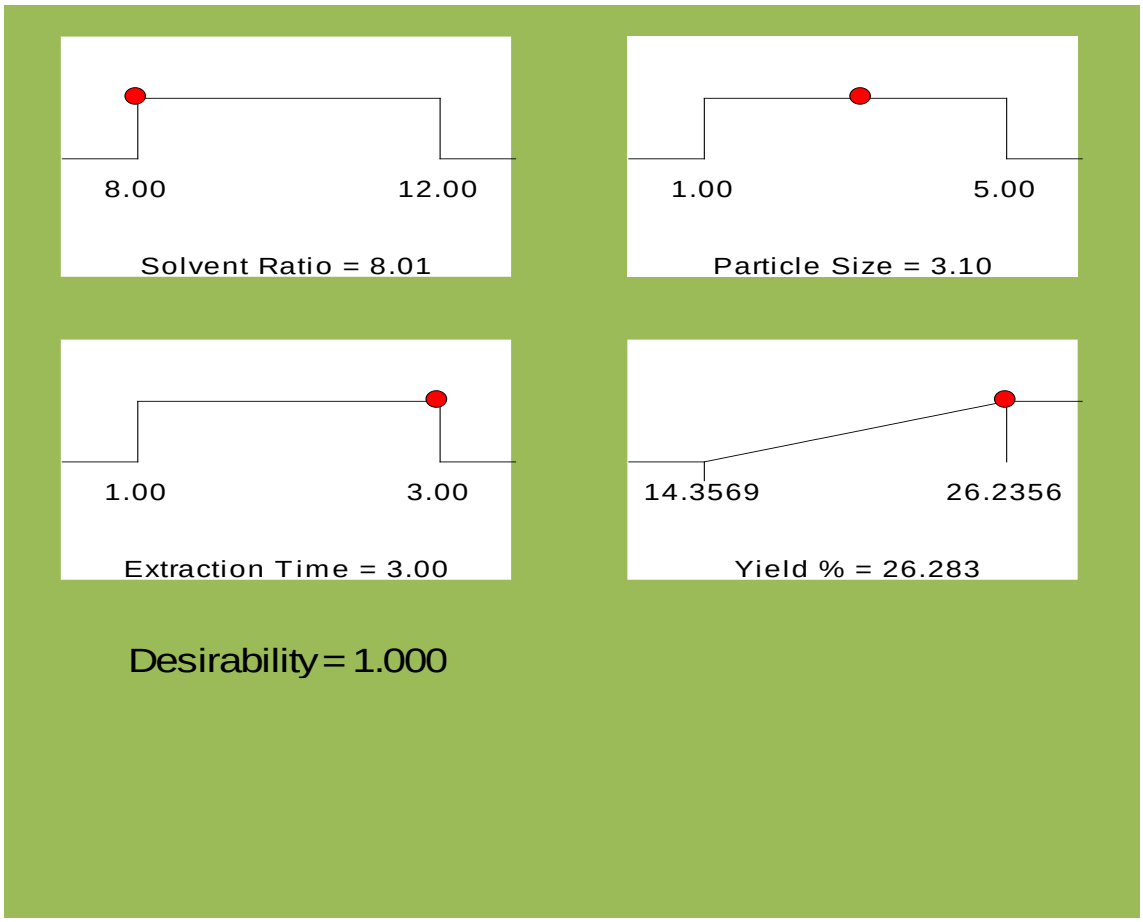
Appendix E: Normal probability plot residuals.



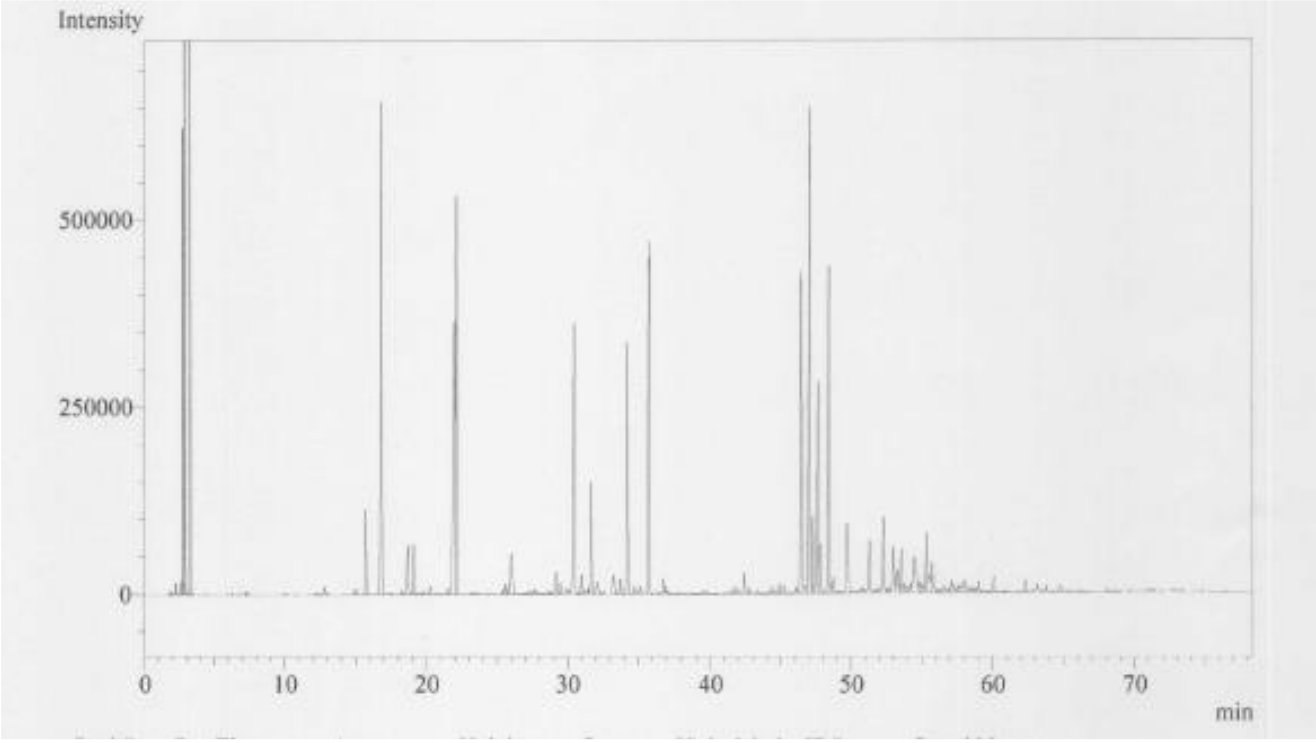
Appendix: F Plot of residual versus predicted



Appendix: G The highest yield offering combination of the treatments



Appendix: H The spectrum chromatogram of extracted ginger oil



Appendix: I 3D response surface method plot of solvent and the particle size