ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

EXTRACTION AND CHARACTERIZATION OF ESSENTIAL OIL FROM PUMPKIN SEED

A Thesis submitted to the school of Chemical and Bio-Engineering Addis Ababa Institute of Technology in partial fulfillment of the requirement for degree of Master of Science in Chemical Engineering under Process Stream.

By: - Meseret Ethiopia

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This is to certify that the thesis prepared by Meseret Ethiopia, entitled: Extraction and characterization of essential oil from pumpkin seed and submitted in partial fulfillment of the requirement for the degree of Master of Science (Chemical and Bio Engineering) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining committee:

	Signature	Date
Advisor: -		
Dr. Eng. Hundessa Dessalegn	_____	_____
Internal examiner		
Dr. Shegaw Ahmed	_____	_____
Extrenal examiner		
Dr. Ing. Abubeker Yimam	_____	_____
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DECLARATION

I declare that this thesis entitled “Extraction and characterization of essential oil from pumpkin seed" has not been submitted in any form for another degree, diploma or an award at any university or other institution of the tertiary education. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and discussions. Information taken from published and unpublished work of others has been acknowledged in the text and a list of references is given. The work was under the guidance of Dr.Eng. Hundessa Dessalegn (Assistant Professor) instructor in Addis Ababa University, School of Chemical and Bio Engineering.

Name: Meseret Ethiopia

Signature: _____

Date: _____

ABSTRACT

The objective of this thesis is extraction and characterization of essential oil from pumpkin seed cucurbita pepo variety using ethanol as solvent. The extraction was carried out using Soxhlet method and separated from solvents by using rotary evaporator. Pumpkin seeds were analyzed for proximate analysis and found to; 5.21 ± 0.12% ,22.5± 0.31%, 50.5± 0.112%, 7±0.09%, 4.5± 0.19% and 10.37±0.079% of moisture, crude proteins, crude fat, crude fiber, ash content, carbohydrate respectively. A full factorial design was applied to pumpkin seed cucurbita pepo variety, and 30 experimental runs were performed followed by optimization of pumpkin seed oil. This work was deals with random experimental design consisted of extraction time from 2 to 6 hours, average particle size from 0.25 to 2 mm, solvent to pumpkin seed meal ratio from 0.05 to 0.1mg/ml under constant temperature (80^{o}c) was done and it was conducted with six center points and three replications. Physiochemical property of extracted oil were specific gravity, pH, kinematic viscosity, density moisture and volatile contents, refractive index, free fatty acid, iodine, acid value and saponification value were: 0.91178, 5.16 ± 0.1, 35, 911.78, 0.07, 1.468, 0.62±0.61, 97.5 I_2g/100g oil, 1.23± 0.23mg KOH/g oil and 189.8±0.31mg KOH/g oil respectively which are in range of literature. FT-IR analysis shows the presence functional group of carbohydrate, carbonyl, alkene, aromatic, alkane, aliphatic amine, carbocyclic, alcohol and methyl ester which is close to essential oil reported in literature. Gas - chromatography-Mass spectroscopy analysis showed the presence of five major free fatty acids linoleic acid, oleic acid, palmitic acid, stearic acid, and miystiric acid, with respective value 57.5%, 20.2%, 19.1%, 2.97% and 0.23%. The pumpkin seed oil also showed good antimicrobial activity against both S. aureus and E.coli with maximum zone of inhibition 15.5±0.23 mm and13.5±0.51 mm respectively. Therefore, it is feasible to be used as edible oil, antimicrobial and other applications.

***Key words**: Essential oil, Pumpkin seed, Pumpkin seed oil, cucurbita pepo, free fatty acid*

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ACRONYMS

AACS	American Analytical Chemical Society
AAIT	Addis Ababa Institute of Technology
AAU	Addis Ababa University
ANOVA	Analysis of Variance
AOAC	Association of official analytical chemists
AV	Acid Value
FAO	Food and Agricultural Organization of the United Nation
EFFA	Essential Free Fatty Acid
FFA	Free Fatty Acid
FT-IR	Fourier Transform Infrared
GC-MS	Gas Chromatography with Mass Spectroscopy
MUFA	Monounsaturated fatty acids
N	Normality
P.S	Particle Size
PUFA	polyunsaturated fatty acid
SFA	Saturated fatty acid
S.G	Specific Gravity
RSM	Response surface methodology
I.V	Iodine value
S.V	Saponification value

1. INTRODUCTION

1.1. Background

Essential oils are aromatic liquids which are extracted from the flowers, seeds, leaves, and stems, bark roots of trees, herbs, bushes & shrubbery through different extraction methods. They were originated in ancient Rome, Greece, and Egypt and throughout the Middle and Far East had, as a common feature, They were used for many purpose such as perfumes, cosmetics, lotion, food preservation, food flavors, deodorants, pharmaceuticals, medicinal and embalming antiseptic There are several techniques that can be used to extract essential oils: water distillation, steam distillation, solvent extraction, expression under pressure, supercritical fluid extractions and subcritical water extractions (Properzi, et al., 2013).

Essential oils are involved in international market and most industries. They are used as industrial raw materials in twentieth century distillation technology. Because of increasing numbers and types of individual oils from day to day, all researcher including pharmacists, chemists and physicians were studying about the physical, chemical, and medicinal properties of oils since in 1550. Their complex mixtures of chemical compounds can be separated and the individual components used as building blocks to introduce a particular flavor or aroma into a product. Currently, the raw materials for essential oil are cultivated by farmers who are found in rural area. They are use the raw material for essential oil for different purpose. From those, different types of essential oils pumpkin seed are a common essential oil known many years ago (Balami, 2007).

Recently, due to high world population growth as well as shortage of food resources, there has been a growing interest in recognition and utilization of new natural and available food materials. One of these foods is pumpkin seeds which attracted many studies in recent years. Pumpkin seed is a seed which obtained from pumpkin. Pumpkins; squash, and gourds are closely related members of the Cucurbit, it is an annual plant growing in temperate and subtropical regions and belongs to the family of Cucurbitaceae which comprises Cucurbita pepo, Cucurbita moschata, Cucurbita maxima, and Cucurbita mixta, according to the texture and shape of their stems. Cucurbitaceae used as vegetable and medicine throughout the world, Which includes approximately 90 orders and 750 species, The species of squash pumpkins is one of 5 cultivated

and about 10 wild species of the genus Cucurbita L. of family 'Cucurbits' Cucurbitaceae (Kwiri et al., 2014; Malley, 2008). All world contents are produce pumpkin except Antarctica including the United States, Canada, Mexico, India, and China. The oldest evidence, pumpkin-related seeds were found between dating 7000 and 5500 BC, in Mexico. Pumpkin is contain several components such as stem, tendril, leaves, lid, pumpkin shell, pulp, ribs, blossom end, cavity, brains, seeds, seed coat nut. Pumpkin fruit is physically composed of seed (Jafari, et al., 2012).

Pumpkin seeds, also known as pepitas, which means are edible and nutrient-rich. They are about 1.5 cm (0.5 in) long and an average of 0.22 grams weight, flat, asymmetrically oval, light green in color and usually covered by a white husk, although some pumpkin varieties produce seeds without them (Srbinoska, et al., 2012). Pumpkin seeds are a popular snack that can be found hulled or semi-hulled at most grocery stores and a good source of protein, magnesium, copper and zinc. The pumpkin seed is valued in relation to its nutritional points. Pumpkin seeds serve as a rich source of edible oil that has a wealthy amount of unsaturated fatty acids and vitamin E, which plays an important role in human health, and both bring a lot of benefits. Noted that the oil content of pumpkin seeds is about 50%, and the four dominant fatty acids are palmitic, stearic, oleic and linoleic acids (Stevenson et al., 2007).

Pumpkin seed oil has been used traditionally as medicine in many countries such as China, Yugoslavia, Argentina, India, Mexico, Brazil, and America. Pumpkin seed oil also rich in antioxidants and beneficial as nutritional supplements such as essential fatty acids including linoleic and linolenic, carotenes, lutein, gamma and P-tocopherols, phytoserols, chlorophyll, selenium and zinc (Rodríguez-Miranda, et al., 2014). Pumpkin seed oil contains essential fatty acids that help to maintain healthy blood vessels, nerves and tissues. The medicinal properties of pumpkin seed oil include anti-diabetic, antioxidant, anti-carcinogenic, and anti-inflammatory. Phytosterols presence in pumpkin seeds is useful for lowering cholesterol and enhancing heart health and reducing the risk of heart (Srbinoska et al., 2012). Pumpkin seeds also contain the compound tryptophan which is necessary to battle the feelings of depression and hypercholesterolemia which leads to cardio vascular disease The elevated levels of serum testosterone may be one of the mechanisms underlying the effect of squalene in pumpkin seeds on improvement in libido and semen quality and the reduction in serum

leptin (Popa et al., 2010). It also improves palatability; enhance the digestibility and nutritive value increase sperm cell count, assisting with fertility challenges, impedes the formation of kidney stones, especially good for those with a history of kidney stones (Abdel-Rahman, 2006).

Pumpkin seed oil and seeds are also rich in unsaturated fatty acids. Due to high omega-3 (6 and 9)-fatty acids. Seeds and oil have been claimed to promote HIV/AIDS wellness. The lignans and phytosterols such as delta 7-sterols and delta 5-sterols are of special interests. Antioxidative compounds, such as vitamin E, especially gamma-tocopherol are also high. In fresh dried seeds concentration of alpha-tocopherol is 37.5 μg/g and gamma tocopherol is 383 μg/g (Bavec, et al., 2007).

Producing essential oil from pumpkin seed industrially will give a quality product and is a major source of income by exporting it to other countries. The extraction of pumpkin seed can be processed through solvent extraction. Solvent extraction is a new alternative technology which has many advantages in relation to traditional methods of fatty compounds extraction vegetable raw materials. Solvent extraction method use ethanol as a solvent which is not toxic and easy to recover fully which results without chemical residue problem. Besides it takes short time for extraction and higher efficiency when compared with other methods.

The aim of this thesis is extraction, characterization and optimization of essential oil from pumpkin seed using ethanol as solvent. Proximate analysis of pumpkin seed, characterization of pumpkin seed oil for different chemical and physical properties and investigate the effects of particle size, extraction time and pumpkin seed meal to solvent ratio with constant temperature in to the yield of essential oil.

However, Ethiopia is a home for the production of many oilseeds, the oilseed essential oil with solvent extraction of pumpkin seed has not yet been produced in the country. In Ethiopia, pumpkins are widely grown fruit which are used effectively and efficiently for stew preparation in order to be consumed with Ethiopian traditional spongy thin-layer bread *(*injira*)* made from cereal grain called teff. Traditionally pumpkin seed are consumed either roasted or raw and used in cooking and baking as an ingredient of cereals, bread, cakes and salads. Currently, Ethiopia export enormous amount of pumpkin seed.

1.2. Statement of the Problem

Ethiopia is located around the tropical region, thus, the weather makes a suitable environment for the growth of pumpkin. There are a lot of pumpkin producing areas such as Gambiella Region, Jijiga, Dire-Dawa, Kulubi Harar and other low land area. Most Ethiopian people use pumpkin as house holding spice stew (wat) preparation. From the literature, it is observed, World Science approves the application of pumpkin seed for medicinal purpose for internal as well as external treatment problems.

Unlike its medicinal purpose, pumpkin seed oil is also used for production of soap, perfumes and lotions, food flavorings, food preservation, nutraceuticals, pharmaceuticals and cosmoceuticals. Because of processing essential oil industrially is very limited in Ethiopia, the amount of imported essential oil is increased from day to day. More than 700 tons of essential oils was annually imported (minimum 350 Million birr) (Addis Ababa Development Study Associates (2008)) ≈ 18.42 USD, In 2012 it reached more than 1800 tones (Custom Authority)≈900 million birr≈46.15 Million USD annually (Martha & Gutierrez, 2016). Nowadays a large amount of pumpkin seed is exported to China, the Middle East and Turkey; instead of exporting the seed, exporting the oil will bring more income and evaluation of the oil yield. Ethiopia should produce pumpkin seed based essential oil in quality and will be a source of income by exporting it to other countries.

1.3. Objectives

1.3.1. General Objective

The aim of this Thesis is extraction, characterization and optimization of essential oil from pumpkin seed using ethanol as a solvent.

1.3.2. Specific Objectives

1. To determine the proximate composition analysis of pumpkin seed (cucurbita pepo)
2. To extract essential oil and conduct physicochemical and qualitative analysis of essential oil produced from pumpkin seed by GC-MS
3. To evaluate the yield and study the functional group of essential oil from pumpkin seed for antimicrobial activity by FTIR
4. To optimize different operational parameters such as extraction time, particle size and pumpkin seed meal to solvent ratio of extracted essential oil using ethanol as solvent

1.4. Significance of the Study

This study is an experimental study of solvent extraction method. Extraction, characterization and optimization of essential oil using pumpkin seed as raw material.

- ✓ This study is contributing a significant method of production of high quality pumpkin seed essential oil using ethanol as solvent.
- ✓ This thesis will show the possibility of extraction technology of pumpkin seed essential oil.
- ✓ To show health benefit of pumpkin seed essential oil.
- ✓ Increase the foreign currency by reducing the raw seed export rather to will send essential oil form.
- ✓ To evaluate the essential oil from pumpkin seed for antimicrobial activity

1.5. Scope of the study

This thesis focused on extraction, characterization and optimization essential oil from pumpkin seed using ethanol as solvent. Pumpkin seed is a seed which obtained from pumpkin. Pumpkins are squash and gourds closely related members of the Cucurbit. The methodologies that were used in this work were solvent extraction flowed by rotary evaporator. The statistical data was generated from laboratory experiments and using analysis of variance (ANOVA) the data were analyzed the effect of process parameters on the percentage oil yield of pumpkin seed and to draw a generalizing conclusion for each parameter on the percentage oil yield of pumpkin seed. Qualitative and quantitative of analysis of extracted pumpkin seed oil were analyzed and evaluation of extracted pumpkin seed oil for antimicrobial activity was also done.

2. LITERATURE REVIEW

2.1. Essential oil

Essential oils are subtle, concentrated, hydrophobic, aromatic liquids containing volatile compounds which is extracted from the flowers, seeds, leaves, stems, bark &/or roots of trees, herbs, bushes & shrubbery through different extraction method. They were recovered in Egyptians, Romans, and Greeks between 1500 B.C to 377B.C. The Bible story also refers to essential or anointing oils over 150 time, which means "to smear with oil", to make a person sacred, to set them apart & to dedicate them to serve a higher spiritual purpose. Essential oils are used in the manufacture of high quality perfumes and lotions, food flavorings, cooking, cleaning, skincare, hair care, massage, aromatherapy, cosmetics, homemade beauty and cleaning products, healing and medicinal purposes and as fragrant seed and antiseptic additives in many common products. Besides essential oils promote hormonal balance, improve digestion and cure respiratory problems and infections *(*Suryawanshi & Kumbhar, 2016).

Essential oils contain the true essence of the plant it was derived from and are highly concentrated. Essential oil is volatile oil, ethereal oils or aetherolea, or simply as the "oil of" the plant from which they were extracted, usually having the characteristic odour or flavor of the plant from which it is obtained, used to make perfumes and flavorings. Oil is essential in the sense that it carries a distinct scent, or essence of the plant. Essential oils are not the same as perfume or fragrance oils. Where essential oils are derived from the true plants, perfume oils are artificially created fragrances or contain artificial substances and do not offer the therapeutic benefits that essential oils offer. Formerly, essential oils are produced by tedious hand pressing and sponge pressing. They are now produced by high-speed machines. The yield of essential oils varies widely from species to species (Vanhaelen et al., 2002).

2.2. Pumpkin

Pumpkin is one of the most important crops of family Cucurbitaceae. Cucurbitaceae used as vegetable and medicine throughout the world (Verla et al., 2014). The word pumpkin originates from the word pepon, which is Greek for "large melon", something round and large. Pumpkin cultivars may belong to one of several species: Cucurbita pepo, Cucurbita maxima, Cucurbita moschata, and Cucurbita mixta (Kaiser & Ernst, 2014). It has been suggested that it has more cultivated forms than any other crop. All world continent produce pumpkin except Antarctica: including United States, Canada, Mexico, India, and China. The oldest evidence, pumpkin-related seeds dating between 7000 and 5500 BC, was found in Mexico, This plant is native of Northern Mexico and southwestern and eastern USA (Gohari et al., 2011).

Natural conditions in Croatia allow an extraordinary successful cultivation of pumpkins used for edible oil production. It is possible to achieve a yield of fruit as high as 80 t/ha. The pumpkins contain about 90% of water and 2 % of seed and the 1000-seed weight is about 200 g. Hull seeded pumpkin seeds contain about 31 % of oil whereas the hull-less pumpkin seeds contain 52 % of oil (Sito & Barc, 2005). These are characterized by a low content of fat (2.3%, pumpkin pulp is not a rich source of oil), carbohydrates (66%), proteins (3%), and by a high-carotenoids content with values of 171.9 to 461.9 µg.g-1 (petal, 2013). Food value per 100 g is: Calories 80 kcal, crude fiber 11.46%, ash 16%. The mineral analysis indicated that pumpkin pulp contained high levels of Mn, Fe, Cu, Pb, P, Ni, Ca, Mg, Na and K. The level of Pb, and Cu are within the acceptable range to FAO (Martha & Gutierrez, 2016). These families have medicinal and nutritional benefits. The immature fruits are consumed as a vegetable. The mature fruit is sweet and used to make confectionery, beverages are roasted, or cooked and can be incorporated into baked goods. The seeds, rich in oil, also are used in Mexico, with honey to prepare desserts known as palanquetas. Flower buds and flowers are also edible in Mexico to prepare quesadillas. Some fruit varieties are used with decorative purposes in Halloween party (Napier, 2009 ; Polk, et al., 2015).

Figure: 2.1 Freash pumpkin fruit plant and matured pumpkin frut; source: Napier, (2009)

2.2.1. Component of pumpkin

The pumpkin is made up of many different parts which give us a perfect fruit once it has matured. It contain several components such as stem, tendril, leaves, lid, pumpkin shell, pulp, ribs, blossom end, cavity, brains, seeds, seed coat nut. Stem is located on the very top of the pumpkin. Brown to brownish green in colour and slightly curved; it is attached to the vine and provides nutrients to grow the fruit, just like an umbilical cord. Tendrils are green, thin and hair-like structure; while the plant is growing, the tendrils twist around objects on the ground to help anchor the vine and protect it from the elements, like the wind. Leaves of the pumpkin absorb energy from the sun to allow the plant and fruit to grow. When you cut a pumpkin (for carving) around the stem to open it, lid is found. Pumpkin Shells refers to both the skin and the pulp of the fruit. The external layer of the pumpkin is called the skin, or rind. This is a protective layer that keeps insects and disease out of the fruit. Pulp is also known as the meat of the fruit which is used to cook with. Ribs are the external shape of the pumpkin which made up of indented ridges running from top to bottom. When the fruit is young a flower blossom is at the end of the fruit. This is known as the blossom end, which becomes the bottom of the fruit. As the female flowers become pollinated a fruit develops and the flower dies off. Fibrous Strands Better known as brains, this part of the fruit consists of its fibrous strings and seeds. Once the fibrous strings and seeds are removed, you are left with the empty cavity of the fruit. Seeds are located in the center of pumpkin and attached to the fibrous strings. The seeds can be separated, dried and eaten, or used for the next harvest. Seed Coat is the outer layer of the seed which helps to protect the nut inside that will eventually grow into a pumpkin plant. This is also known as the seed jacket. Nut is located inside of the seed. When a seed is planted the moisture and warmth triggers the nut to

begin to grow into a new plant. Pumpkin fruit is physically composed of seed("Agriculture , Forestry," 2009).

2.3. Pumpkin Seed

Pumpkin seed (Cucurbita pepo) is high in oil, protein, and total unsaturated fatty acids (TUFA) and provides an important source of nutrition and income globally (Meru, et al., 2017). Pumpkin seeds, are flat, asymmetrically oval, light green in color and usually covered by a white husk and used as edible, although some pumpkin varieties produce seeds without husk. Pumpkin seeds are also snack that can be found hulled or semi-hulled at most grocery stores. Pumpkin seeds are a good source of protein, magnesium, copper and zinc**.** The pumpkin seed is valued in relation to its nutritional points. The four fatty acids present in significant quantities are palmitic, stearic, oleic, and linoleic acids (Adepoju & Adebanjo, 2011; Abuelgassim & Arabia, 2012).

Figure: 2.2. Hulled pumpkin seed ; Source **:** Polk, et al., (2015)

The pumpkin seed is valued in relation to its nutritional points. The four fatty acids present in significant quantities are palmitic, stearic, oleic, and linoleic acids (C. M. et al., 2012), reported that pumpkin seed is a nutritious food with a high oil (50% w/w) and protein (35%) content that varies depending on cultivar Triterpenoids including 0.08-0.2% of multiflorane p-aminobenzoates. Pumpkin seeds also contribute to a good amount of essential

fatty acids Omega-6 and Omega-9. The pumpkin seeds possess dietary and medicinal qualities besides being the source of good quality edible oils (Borhade, 2014).

2.3.1. Constituents of Pumpkin seed

The physical-chemical characteristics of the oil and its content of fatty acids, tocopherols, carotenoids, chlorophyll pigments, squalene and sterols are described in the literature (Moo-huchin et al., 2013). The fatty oil content of pumpkin seed is about 50% (45-60%). Fatty acids 9% saturated fatty acids, 34% monounsaturated fatty acids, 55% (double) polyunsaturated fatty acids, 2% (triple) polyunsaturated fatty acids. Pumpkin seed oil contains a high amount of the essential fatty acids linoleic acid and linolenic acid (Bavec, et al., 2007). Whereas linoleic acid accounts for nearly one-third of the total fatty acid in pumpkin seeds, only Squalene: Squalene (39-46%) is the characteristic constituent of the unsaponifiable fraction of the oil seeds. It can be used as a marker for the differentiation of oils obtained from other seeds. Tocopherols ca. 360-540 mg/kg of oil, comprise β- and γ- (but not α-) tocopherols. Pumpkin seeds were found to have the greatest content of tocopherols (16 mg/100 g) with β- and γ-tocopherol being predominant over α-tocopherol. Carotenoids, mainly lutein (50%) and β-carotene (10-12%) with smaller amounts of cryptoxanthinn and race amounts of α- and γ-linolenic acids were found. Minerals and trace elements: chlorine, iron, fluorine, iodine, potassium, calcium, copper, magnesium, manganese, sodium, phosphorus, selenium, sulfur, zinc. Proteins are abundantly present in the seeds (31% - 51%) such as a high content of aspartic acid, glutamic acid and arginine and low lysine content. Carbohydrate content is between 6% and 10%. Vitamin A as beta-carotin, Vitamin E (50 mg per 100 ml), B1, B2, Vitamin C, Niacin, Niacin equivalent, pantothenic acid, pyroxene, biotin (Nadu, 2018**).** The Proximate composition of pumpkin seed (cucurbita pepo) are summarized in the Table: 2.1.

Table: 2.1 proximate compositions of pumpkin seed (cucurbita pepo) in percentage

Components	Value
Moisture content	5 %
Crude Ash	4.9%
Crude Fiber	6%
Crude Protein	30.23%
Crude Fat	49.01%
Carbohydrates	4.89%
Energy	559 Kcal

Source: USDA National Nutrient database

2.4. Pumpkin in Ethiopia

Ethiopia is located around the tropical region; the weather makes a suitable environment for the growth of pumpkin. Few years ago, farmers used to produce pumpkin in their gardens together with cereals, in farms near fences for the plants to easily creep on fences and houses, marginal or waste land, on decaying hay and heap of cow dung. There are wide variations in fruit size, weight, shape, color, vine length and branching, leaf size, overall fruit subjective and chemical qualities and seed size among varieties. In Ethiopia, pumpkins are widely grown for their fruit utilization that are effectively and efficiently being used for stew preparation to be consumed with Ethiopian traditional spongy thin-layer bread *(*injira*)* made from cereal grain called teff. The other most important food product from pumpkin fruit is dried pumpkin which is called 'Duba quanta*'*. This dried product is used for making delicious stew called *'*Duba Wett' and highly used during the period of food scarcity. Pumpkin is a seasonal crop that has been used for human food (Workineh, et al., 2014).

Recently there are a lot of pumpkin producing areas such as Gambiella Region, Jijiga, Dire-Dawa, Kulubi Harar and other low land area. Most commonly cucurbita pepo Varieties are known and grown in Ethiopia. It is sensitive to frost and ideal temperature of 18^0c to 30 ^{0}c best for growth. Varieties of C. pepo germinate best with eight hours of sunlight daily and a planting depth of 1.2 centimeters (0.47 in). Seeds planted deeper than 12.5 centimeters (4.9 in) are not likely to germinate. In a seed batch with 90 percent germination rate, over 90 percent of the plants had sprouted after 29 days from planting. Most pumpkins reach maturity at 3 months to 4

months after sowing. The maturity can be identified by change in fruit colour to orange or pale yellow colour. At this stage, the pumpkin seed attain maturity with higher vigour and viability. The yield depends on many factors which may include soil type, cultivar, irrigation, age of plants and management practices. However, an average yield may range 15 to 25tonnes/ ha. Pumpkins generally weigh between 3 and 8 kilograms (6 and 18 lbs.), though the largest cultivars (Workineh, et al., 2014).

2.5. **Uniqueness of essential oils**

In early work, the term "essential oils" was defined as the volatile oils obtained though different extraction method from plants. This definition was clearly intended to make a distinction between "fatty oils" and the oils, which are easily volatile. Gradually with the advance of science came improvements in the methods of preparing the oils, and parallel with this development a better knowledge of the constituents of the oils was gained. It was found that the oils contain many classes of organic substances with varying volatility. Although a list of all the known oil components would include a variety of chemically unrelated compounds, it is possible to classify these into four main groups of essential oils (Balami, 2007).

- ✓ Terpenes, related to isoprene
- ✓ Straight-chain compounds, not containing any side branches
- ✓ Benzene derivatives
- ✓ Miscellaneous

Essential oils are different from other oils by the following properties:

Volatile

Essential oils are the volatile fragrant components from various indigenous and exotic plants which have been traded internationally for several centuries. All true essential oils are secondary metabolites of plant products and in some instances the oil extracted from one part of the plant is different from that extracted from other parts.

Aromatic

Essential oils are highly aromatic and therefore, many of the benefits can be obtain by simply inhaling them. This can be done by breathing in the fragrance from the bottle, or they can be diff used into the room. Essential oils, when diffused, can be the best air filtration system in the world. They will purify the air by removing metallic particles and toxins from the air, increase atmospheric oxygen, increase ozone and negative ions in the house, which inhibits bacterial growth, destroy mold, cigarettes and animal odors, fill the air with a fresh, herbal aromatic scent.

Penetrating characteristics

The penetrating characteristic of essential oils greatly enhances their ability to be effective. Essential oils will penetrate into the body when applied to the skin. Essential oils rubbed into the feet will be distributed to every cell in the body in minutes. They will even penetrate a finger or toe nail to treat fungal infection underneath.

High frequency

The effectiveness of essential oils is sometimes also described in terms of frequency. It has been reported that the human body has an electrical frequency and that much about a person's health can be determined by frequency. In 1992, Bruce Tainio of Tanio Technology, an independent division of Eastern State University in Cheny, Washington, built the first frequency monitor in the world. Tainio has determined that the average frequency of the human body during the day time is 62-68 MHz. (a healthy body frequency is 62-72). When the frequency drops, the immune system is compromised. If the frequency drops to 58 MHz, cold and flu symptoms appear, at 55 MHz, diseases like Candida take hold, at 52 MHz, Epstein bar and at 42 MHz, cancer. According to Dr. Royal R. Rife, every disease has a frequency. He found that certain frequencies can prevent the development of disease and that others would destroy disease. Substances with higher frequency will destroy diseases of a lower frequency. The study of frequencies raises important questions, concerning the frequencies of substances we eat breath and absorb. Many pollutants lower healthy frequency. Processed canned food has a frequency of zero. Fresh produce has up to 27 MHz Essential oil start at 52 MHz and go as high as 320 MHz, which is the frequency of Rose oil. Clinical research shows that essential oils have the highest frequency of any natural substance known to man, creating an environment in which disease, bacteria, virus, fungus, etc., cannot live (Balami, 2007).

2.6. Classification of essential oils

Essential oils may be classified using different criteria: consistency, origin, and chemical nature of the main components.

2.6.1. Consistency of essential oils

Essential oils are classified depending on their consistency such as, fluid essences, balsams and resin. Fluid essences are liquids which are volatile at room temperature. Balsams are natural extracts obtained from a bush or tree. They usually have a high benzoic and cynamic acid content with their corresponding esters. They are thicker, not very volatile, and less likely to react by polymerizing. Examples of balsams are Copaiba balsam, Peruvian balsam, Banguy balsam, Tolu balsam, Liquid amber. Resin group are find a number of possible combinations and mixes as defined below: Resins, these are amorphous solid or semi-solid products of a complex chemical nature. They are physiological or physio-pathological in origin. Colophony, for example, is obtained by separating trementine an oleoresin. It contains abietic acid and derivate. Oleoresins, these are homogeneous mixes of resins and essential oils. Trementine, for example, is obtained by making incisions in the trunk of different pine species. It contains resin (colophony) and essential oil (trementine essence) which are separated by steam drag distillation. Gum-resins, these are natural plant or tree extracts. They are a mix of gums and resins.

2.6.2. Origin of essential oils

Essential oils are classified depending on their origin such as natural oils, artificial oils and Synthetic oils. Natural oils are obtained straight from the plant and are not modified physically or chemically afterwards. However, they are expensive because of their limited yield. Artificial oils are obtained using processes of enriching the essence with one or several of its components. For example, essences of rose, geranium, and jasmine are enriched with linalool, and aniseed essence with ethanol. Synthetic oils, as the name suggests, are usually produced by combining their chemically synthesized components. These are the cheapest and are thus much more commonly used as fragrance and taste enhancers (vanilla, lemon and strawberry essences).

2.6.3. Chemical nature of essential oils

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, waxes, and flavonoids.

Essential oils consist of chemical compounds that have hydrogen and carbon as their building blocks. Basic Hydrocarbon found in plants is isoprene having the following structure. Terpenes generally have names ending in "ene." For examples: Limonene, Pinene, Piperene, Camphene, etc. Terpenes are anti-inflammatory, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes and diterpenes. Referring back to isoprene units under the Hydrocarbon heading, when two of these isoprene units join head to tail, the result is a monoterpene, when three join, it's a sesquiterpene and four linked isoprene units are diterpenes. Alcohols are the compounds which contains Hydroxyl compounds. Alcohol exists naturally, either as a free compound, or combined with a terpenes or ester. When terpenes are attached to an oxygen atom, and hydrogen atom, the result is an alcohol. When the terpene is monoterpene, the resulting alcohols called a monoterpenol. Medicinally, essential oils containing aldehydes are effective in treating Candida and other fungal infections. Acids are Organic acids in their free state are generally found in very small quantities within essential oils. Plant acids act as components or buffer systems to control acidity. Esters are formed through the reaction of alcohols with acids. Essential oils containing esters are used for their soothing, balancing effects. Because of the presence of alcohol, they are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system. They generally are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system.

2.6.4. Physical properties

Specific gravity

Specific gravity is an important criterion of the quality and purity of an essential oil. Values for essential oils vary between the limits of 0.696 and 1.188 at 15 °C; in general, specific gravity is less than 1.000. Hence essential oil can be collected over (floating on) water.

Refractive index

Refractive indexis the ratio of the speed of light at a definite wave length in a vacuum to its speed in the medium and this varies with the wave length of light and temperature is calculated in equation 2.1.

$$N = \tan \Theta b \qquad (2.1)$$

Where: N= is the index of refraction of the denser medium.

Θb =Angle of refraction, the refractive index of air is 1.

Refract meters of for a rapid and convenient method for the determination of this physical constant.

Boiling range

In the case of isolates and synthetics, the boiling range is an important criterion of purity.

Evaporation residue

An important criterion of purity is the evaporation residue; i.e., the percentage of the oil which is not volatile at 100°C. It is important to study the odor of oil as it volatilizes during the heating.

Flash point

The flash point may prove useful in the valuation of an essential oil. The flash point has value as an indication of adulteration: additions of adulterants such as alcohol and low boiling mineral spirits will greatly lower the flash point.

Solubility

Solubility in alcohol: Most essential oils are only slightly soluble in water and are miscible with absolute alcohol. The solubility of oil may change with age.

Solubility in water: Most of essential oils of commercial interest are steam volatile, reasonably stable to action of heat and practically insoluble in water.

2.6.5. Factors affecting the yield and quality of essential oils

The yield and quality of essential oils have been known to vary due to a number of factors.

2.6.5.1. Particle size

To increase the rate of solvent extraction, it is desirable that the range of particle size to be small. This is due to the greater interfacial areas between the solid and liquid and therefore the higher is the rate of transfer of material (pershat, 2014).

2.6.5.2. Choice of solvent

Use of a suitable solvent for effective separation is very important. Metal chelates and many organic molecules, being essentially covalent compounds do not impose many restrictions on the solvent and the general rules of solubility are of great use. In ion association systems and particularly in oxonium type ions, the role of solvents is very important. This is due to involvement of solvent in the formation of extractable species (Rodríguez-Miranda et al., 2014).

In addition to the consideration of the distribution of the solute in a particular solvent system, the ease of recovery of the solute from the solvent is important for subsequent analytical processing. Thus, the boiling point of the solvent or the ease of stripping by chemical reagents is considered in the selection of a solvent where the choice exists. Similarly, the degree of miscibility of the two phases, the relative specific gravities, viscosity and tendency to form emulsions should be considered. With regard to safety, the toxicity and flammability of the organic solvents must be considered. Sometimes it is possible to achieve the desired characteristics of a solvent by employing a mixed-solvent system. An example of this is the use

of mixtures of alcohols and ethers for the extraction of the thiocyanate complexes of metals. Another method of varying the properties of the extracting solvent is to use organic diluents. Various organic compounds such as kerosene and other hydrocarbons are employed to dilute tributyl phosphate for extraction purposes.

2.6.5.3. Temperature

The solubility of the material which is being extracted will increase with temperature to give a higher rate of extraction.

2.6.5.4. Condition of raw material

Condition of raw material is important because some materials like roots and seeds will not yield essential oil easily if distilled in their natural state. These materials have to be crushed, powdered or soaked in water to expose their oil cells.

2.6.5.5. Effect of solvent to solid ratio

Solvent to solid ratio is another important parameter that affects oil extraction efficiency and recovery. The volume of the solvent should not be more than an optimized volume because the cost of the solvent recovery could be too high, thereby increasing the total operational cost (Baldosano, et al., 2015).

2.6.5.6. Effect of extraction time

Extraction time is essential in economizing energy and cost of the extraction process. This could be explained by the Fick's second law of diffusion witch predicts a final equilibrium between the concentrations of solute in the solid matrix and in the bulk solution after a certain time (Baldosano, et al., 2015).

2.7. Source of natural essential oil

Essential oils are generally derived from plant organs which containing natural essential fatty acids. From one or More plant parts, such as flowers, leaves, stems bark, wood roots seeds fruits , rhizomes and gums or oleoresin are explained in Table 2.3 below. Depending upon the plant family, essential oils may occur in specialized secretary structures such as glandular hairs, modified parenchymal cells (Piperaceae), resin canals (conifers), oil tubes called vittae

(Umbelliferae), lysigenous cavities (Rutaceae), schizogenous passages (Myrtaceae, Graminae, Composite) or gum canals (Cistacae, Burseraceae) (alemu, 2016).

Table: 2.2. Source of natural essential oil

Leaves	Flowers	Peel	Seeds	Wood	roots	Rhizome
Basil	Chamomile	Bergamot	Almond	Camphor	Valerian	Ginger
Bay leaf	Clary	Grape fruit	Anise	Cedar		
Cinnamon	Sage	Lemon	Celery	Rosewood		
Eucalyptus	Clove	Lime	Cumin	Sandalwood		
Lemon Grass	Geranium	Orange	Nutmeg Oil			
Melaleuca	Hyssop	Tangerine	Pumpkinseed			
Oregano	Jasmine					
Patchouli	Lavender					
Peppermint	Manuka					
Pine	Marjoram					
Rosemary	Orange					
Spearmint	Rose					
Tea Tree	Ylang-ylang					
Wintergreen						
Thyme						

2.8. Pumpkin seed oil

The pumpkin seed oil is dark green in color that contains a high amount of free fatty acids including four dominant fatty acids (oleic, linoleic, palmitic and stearic) are present with the relative distribution of 43.8%, 33.1%, 13.4% and 7.8% respectively, representing 98 + 0.1% of the total fatty acids amount. The oil content of dry pumpkin seeds is 47.03%. However, the

variability of the oil contents in various pumpkin species is predominantly attributed to its broad genetic diversity. Importantly, pumpkin seed oil is used as nutritional supplements for natural source of proteins, essential fatty acids, polyunsaturated fatty acids, omega 3, 6 and 9, carotenes, lutein, vitamins such as carotenoids and β- and γ-tocopherols, phytosterols, chlorophyll, and trace elements, such as zinc and selenium. In addition, several triterpenes such as cucurbita-5, 24-dienol, α- and β-amyrin and sterols are present in the seeds and flowers of pumpkin (Jafari et al., 2012).

2.9. Use and application of essential oil

Essential oils are used in several healing systems, including aromatherapy, medicine and massage therapy. Essential oils are used for skin and scalp conditions including acne, burns, cuts, dandruff, insect bites, parasites, warts, and wrinkles. They are recommended for muscle, joint, and circulation problems such as high blood pressure, cellulite (fatty deposit causing a dimpled or uneven appearance, around the thighs and buttocks), aches and pains. For respiratory problems and infections, various essential oils are prescribed for allergies, asthma, earache, sinus infections, colds and flu.

2.10. Property and application pumpkin seed oil

Pumpkin seed contains antioxidants like Beta-carotene. These Antioxidant helps in removing the free radicals from the body, hence it reduces the risk of cancer and other diseases. Beta carotene converts into vitamin-A which strengthens our immune system. Pumpkin seed oil contains essential fatty acids that help maintain healthy blood vessels, nerves and tissues. It also contains Phytosterols, carotinoids, proteins, tocopherols, and phytoestrogenst reduces cholesterol in blood (Meru & Yagiz, 2017; Abdel-Rahman 2006). It enhances the immune response and decrease risk of certain cancers. Pumpkin seed oil hypothesis of the pharmaceutical action is based on the inhibition of 5-a-reductase. The enzyme 5-a-reductase (testosterone 5-a-reductase, EC 1.3.99.5) converts testosterone to dihydrotestosterone (DHT), which is the active male sex hormone. The enzyme is a nuclear membrane bound NADPH-dependentd-3-ketosteroid 5-a-oxido-reductase (5-a-reductase). Pumpkin seed oil has been found useful in the treatment of benign prostatic hyperplasia (Gold, 2009).

Pumpkin seed oil is most commonly used to treat irritable bowel syndrome. Since Pumpkin seeds contain L-tryptophan, which is a compound naturally effective against depression, it is also used for depression treatment (L'Huillier, 2007; Rezk & Darwish, 2012). It has also found to prevent atherosclerosis and regulate cholesterol levels. In German folk medicine, it has been a remedy for parasitic infestations of the intestinal tract such as tapeworm (Shaban & Sahu, 2017). It is composed of unsaturated fatty acids like: myristic, palmitic, Stearic, oleic, Linoleic and Linoleic acid. Pumpkin seed oil is a rich source of antioxidants and polyunsaturated fatty acids. This oil is used for preparation of dessert, ice cream, brittle and soup. It gives a nutty taste to that product. Pumpkin seed oil has moderate amount of saturated fat and good amounts of mono and polyunsaturated fats (Nadu, 2018).

2.10.1. Antimicrobial activity of pumpkin seed oil

Diseases caused by bacteria, viruses, fungi and other parasites are major causes of death, disability, and social and economic disruption for millions of individuals. Despite the existence of safe and effective interventions, many individuals lack access to needed preventive and treatment care. Increasing drug resistance in infectious microorganisms has warranted the development of new drugs against pathogenic micro-organisms. In this regard, natural sources have been considered as the best option to isolate new and novel anti-microbial components. Various broad spectrum anti-microbial components have been isolated from pumpkins. Pumpkin had properties of biological activities varies from antimicrobial to antitumor. The antimicrobial activity of pumpkin has many applications, including preservation, pharmaceuticals, alternative medicine and natural therapies.

2.11. Extraction technologies of essential oils

The quality, flavor and nutritional value of essential oils are directly related to the way the oil is extracted and processed. The highest quality oils are exposed to the least amount of heat, light, pressure and chemicals in the extraction and refining process. The yield and composition of essential oils depends on geographic location and agricultural factors. The extraction of essential oils from plants may be processed by several methods such as steam distillation,

effleurage, Hydro-diffusion, maceration, solvent extraction, enzymatic, micro-wave and supercritical fluid extraction, etc. (blame, 2006).

2.11.1. Solvent Extraction

Extraction essential oil by solvent is recommended if it is necessary to reduce the oil contents in the raw material to lower than 2%. Soxhlet extractor was used to extract oil from solid material by using a different solvent such as hexane, methanol, ethyl acetate, and Aqueous (Badejo et al., 2016). A single component fluid is said to be supercritical when its temperature and pressure both exceed their critical values, without being far from the critical state. At these elevated conditions the properties of the fluid has both liquid and gas properties (Gaspar, et al., 2002).

As solvent extraction uses very little heat, it is found to be advantageous in producing essential oils with whole fragrances that would otherwise be destroyed or altered during steam distillation. Therefore this extraction technique can be used to extract essential oils from very delicate plants to produce higher amounts of essential oils at lower costs. However, some disadvantages associated with the solvent extraction technique. Solvent residues often contaminate the product causing side effects which make the use of essential oil undesirable for skin applications but could still be fine for fragrances or perfumes. Therefore with solvent extraction effective separation of the extracted oil from the solvent is necessary to remove any solvent which may contaminate the essential oils. This process also sometimes yields an aromatic resinous product known as oleoresin which is more concentrated than essential oils with an even wider application in the food and other industries, as discussed by Badejo et al., (2016).

2.11.2. Steam distillation

Most commonly, the essence is extracted from the plant using an technique called distillation. One type of distillation places the plants or flowers on a screen. Steam is passed through the area and becomes “charged” with the essence. The steam then passes through an area where it cools and condenses. This mixture of water and essential oil is separated and bottled. Since plants contain such a small amount of this precious oil, several hundred pounds may need to produce a single ounce (Cassel & Dellacassa, 2008).

Essential oils can be extracted using a variety of methods, although some are not commonly used today. Nowadays, a reputable distiller will try to preserve the original qualities of the plant, but the final therapeutic result is often not formed until after the extraction process. During extraction, the qualities of the oil change to give it more value – for example, chamazulene (characteristic of the pure blue colour of German Chamomile) is formed during the steam distillation process. Currently, the most popular method for extraction is steam distillation. Steam distillation has been used for hundreds of years and today remains one of the most favorably methods of extracting essential oils. Technically speaking if it not extracted using steam distillation or cold expression it is not a therapeutic grade essential oil. A number of factors determine the final quality of a steam distilled essential oil. A side from the plant material itself, most important are time, temperature and pressure, and the quality of the distillation equipment (Shekhar et al., 2013).

2.11.3. Hydro-diffusion

Although introduced more recently than carbon dioxide extraction, hydro-diffusion is similar to steam distillation except that the steam is produced above the plant material and percolates down through it. The advantage of hydro-diffusion over distillation is that the process is quicker, especially for fibrous material such as woods and barks. The resultant oils are reported to have a superior aroma and a richer colour obtained by ordinary distillation. Nevertheless, oils captured by hydro-diffusion process are not widely available (Shekhar et al., 2013).

Many old-time distillers favor this method for most oils, and say that none of the newer methods produces better quality oils. Steam distillation is a special type of distillation or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point. The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point(s) of the individual constituent(s). Essential oils contain substances with boiling points up to 200°C or higher temperatures. In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to 100°Cat atmospheric pressure (Chemat & Boutekedjiret, 2015).

2.11.4. Enzymatic Pretreatment

An interesting method to enhance the oil recovery from the seeds or fruits is the treatment of the raw material by enzymes. Enzymatic Pretreatment, the need of disruption of cell walls by mechanical treatment during flaking is reduced. Additionally, the extraction time decreases, which increases the efficiency of the pressing process for 92%. Further on, this procedure has a lower energy requirement and results in less losses of hexane during solvent extraction (Matthäus, 2012).

2.11.5. **Cold Pressed**

Another method of extraction essential oils is cold pressed expression, or scarification. It has been reported to be used mainly to obtain citrus fruit oils such as bergamot, grapefruit, lemon, lime, mandarin and orange oils. In this process, the fruit is rolled over a through with sharp projections that penetrate their peels thereby piercing the tiny pouches containing the essential oil. The fruit is then pressed to squeeze out the juice from the pulp thereby releasing the essential oils from the pouches. The essential oils rise to the surface of the juice and are separated by centrifugation. Cold pressing is more competitive for specific raw material than methods such as supercritical fluid extraction as it is extremely fast, cheap and does not pollute the extracts, although it does not provide a way of selectively extracting (Balami, 2007).

2.11.6. **Microwave**

The raw material is heated directly by microwaves and this brings about quality consistency and minimizes the impact on the environment as opposed to using fossil fuels or less efficient, indirect electrical heating systems. Specifically in the essential oil extraction microwave mediated processes are highly desirable due to their small equipment size (portability) and controllability through mild increments of heating. However, so far the microwave technology has found application in very few industrial bio-processing installations. Due to the lack of available data on microwave interaction with heterogeneous natural raw materials. The sensing and close control of microwave process is a challenging science and there seems to be insufficient literature in this regard (Gomez & Witte, 2001).

2.11.7. Supercritical fluid

Considering that the supercritical CO2 extraction is a new alternative technology which has many advantages in relation to traditional methods of fatty compounds extraction from vegetable

raw materials(Mohameda & Mansoorib, 2002). The low humidity of pumpkin seeds (6.3%) is an advantage in validity terms, and in the feasibility of lipid substances extraction with supercritical carbon dioxide. The extraction of vegetable oils using supercritical carbon dioxide has been studied as a potential alternative to the current industrial process of expeller pressing, prepress solvent extraction and straight liquid solvent extraction. Supercritical carbon dioxide extraction is the process of separating one component from another (the matrix) using supercritical carbon dioxide as the extracting solvent. It is also referred as "dense gas/phase extraction" or supercritical fluid extraction (Sapkale & Bhatbhage, 2010). Carbon dioxide is the substance most commonly used for supercritical processes because of its easy-to-reach critical temperature and pressure, its chemical stability, non-flammability, non-toxicity, low cost, stability under radioactive conditions, and the easy recovery of extracts (Tongnuanchan & Benjakul, 2014).

2.11.8. Effleurage

Some flowers, such as jasmine or tuberose, have such low contents of essential oils and are so delicate that heating them would destroy their blossoms before releasing the essential oils. In such cases, an expensive and lengthy process called effleurage is sometimes used to extract the essential oils. As described in the literature (Stahl et al., 1988), flower petals are placed on trays of odorless vegetable or animal fat, which absorb the essential oils from them. At the end of every day or even after a few hours, when the vegetable or fat has removed as much of the essential oil as possible, the depleted petals are removed and replaced with fresh ones. This procedure is repeated until the fat or oil becomes saturated with the essential oil. Adding alcohol to this effleurage mixture separates the essential oils. This method employs a similar operating principle and technique to what was discussed for solvent extraction.

2.12. Essential oil from pumpkin seed

Pumpkin (cucurbitae family) locally known as Duba: It is good source of nutritionally essential components. The oil extracted from the extraction of pumpkin seed is used as essential oil. Extraction of pumpkin seed oil using solvent extraction method is discussed as follow.

2.12.1. Solvent extraction of pumpkin seed

2.12.1.1. Selection of the solvent

The solvent for extraction has to withdraw the active agent from a mixture. solvent has high in order to extract the active agents and to reduce the amount of necessary solvent the capacity of the solvent has to be high. To achieve simple regeneration of solvent the miscibility of solvents and primary solvents has to be low. Difference in density in also has great contribution for separation of two phase liquids by using density difference. Optimal surface tension, low surface -> low amount of energy for dispersing required; if surface tension < 1 mN/m stable emulsions are produced. Surface tension > 50 mN/m -> high amount of energy for dispersing and high tendency to coalesce. Solvent recovery, the solvent has to be separated from the extracted phase easily to produce solvent free active agents. The solvent must be non-corrosive prices for construction increases. Solvent must has low price and no or low toxicity. Flame temperature is 25^0c higher than operating temperature. Vapour pressure, to prevent loss of solvent by evaporation a low vapour pressure at operating temperature is required. Low viscosity of the solvents leads, to low pressure drop, good heat and mass transfer.

2.12.1.2. Chemical and thermal stability of solvent

There is no ideal solvent, which fulfills these entire requirements. There are different solvents used for the extraction of pumpkinseed; benzene, hexane, ethanol and petroleum ether are some of the solvents. Benzene has a high boiling point (80.1^0C) resulting high amount of benzene in the last product. Besides, benzene is highly flammable. Therefore, benzene is not preferable solvent for the extraction of pumpkin seed.

Hexane which has a moderate boiling point (69^0C) is relatively expensive when compared with benzene and toxic so it is not prefer for extraction of pumpkin seed oil. Petroleum ether is the best solvent, unfortunately it is very costly and is not found easily as a result it will not be an option as solvent for the extraction of pumpkin seed. Ethanol has also moderate boiling point about (78^0c) and relatively cheap when compared with hexane and benzene. And also it is not toxic; hence ethanol is a preferred for the extraction of pumpkinseed oils.

2.12.2. Physical and chemical property of ethanol

Ethanol is a clear colorless, flammable solvent with a boiling point of 78.5°C; also known as ethyl alcohol, grain spirits, or alcohol. Unlike other alcohols of similar molecular weight, ethanol is considered non-toxic or a drinking alcohol. Ethanol is a polar solvent that is water-soluble

and has a 55°F flash point. Ethanol has a vapor density of 1.59, which indicates that it is heavier than air. Consequently, ethanol vapors do not rise, similar to vapors from gasoline which seeks lower altitudes. Ethanol's specific gravity is 0.79, which indicates it is lighter than water but since it is water-soluble it will thoroughly mix with water. Ethanol has an auto-ignition temperature of 793°F and a boiling point of 173°F. Ethanol is less toxic than gasoline or methanol. Ethanol's greatest hazard as a motor fuel component is its flammability. It has a wider flammable range than gasoline (LEL is 3.3 percent and UEL is 19 percent) (Tangka, et al., 2011).

In a pure form, ethanol does not produce visible smoke and has a hard-to-see blue flame. In a denatured form there is little to no smoke, but a slight orange flame may be visible. Interestingly, ethanol and some ethanol blends can conduct electricity Ethanol found in transportation fuels has been denatured, generally by the addition of up to 5 percent gasoline, rendering it unfit for drinking and thereby avoiding the tax burden imposed on liquor by the Alcohol and Tobacco Tax and Trade Bureau, formerly known as the Alcohol Tobacco and Firearms (ATF). It is a renewable fuel source that is produced by fermentation and distillation process. The most common source of ethanol in the United States in 2008 is corn. However, it can be produced from other products such as sugar cane, saw grass, and other natural products that will be conducive to the fermentation/distillation process (Tangka, et al., 2011). Physical and chemical property of ethanol is summarized in table: 2.2 below.

Table: 2.3. Physical and chemical property of ethanol

Property	**value**
Flash Point	55°F
Ignition Temperature	793°F
Specific Gravity	0.79
Vapor Density	1.49
Vapor Pressure	44 mmHg
Boiling Point	173°F
Flammable Range (LEL–UEL)	3.3%–19%
Conductivity	Yes

Smoke Character	Slight to none
Toxicity	Lower than methanol
Solubility	higher

Reference: The National Institute for Occupational Safety and Health (NIOSH) Pocket Guide to Chemical Hazards

2.12.3. Soxhlet Extraction

The Soxhlet method is the most commonly used semi-continuous process for the extraction of lipids from foods. According to Soxhlet procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent, usually hexane or petroleum ether, ethanol. The ethanol was used for the purpose of solvent. The grounded pumpkin seed samples were placed in a filter paper. The filter paper is then placed in an extraction chamber which is being suspended above a flask containing the solvent and below a condenser.

Heat is being applied to the flask and the solvent evaporates and moves to the condenser where it is converted into liquid that trickles in to the extraction chamber containing the sample. The extraction chamber is made in such a way that when the solvent surrounding the sample exceeds a certain level it over flows and trickles back down in to the boiling flask. The flask containing solvent and lipid is removed at the end of the extraction process. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the round bottom 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 non-soluble portion of the extracted solid remains in the thimble, and is usually discarded. The mixture of solvent and essential oil has been separated by means of a rotary evaporator, and then the essential oil was used for further characterization of analysis and other applications but solvent has been used other extraction process (recycling) operation.

3. MATERIALS AND METHODS

3.1. Materials and Equipment

Materials and chemicals used during the experiment were pumpkin seed, hexane (99.9%),ethanol (99%), sodium hydroxide (99%), 95% ethanol, potassium hydroxide (85 %), hydrochloric acid, H_2SO_4, saturated sodium carbonate, acetone, phenolphthalein, filter paper distilled water, Mueller Hinton agar, Mueller Hinton broth, DMSO, alcohol and Staphylococcus aureus and Escherichia coli bacteria.

The equipment used were: Soxhlet set up, water bath, chiller, crucibles, burette, centrifuge, condenser, oven, viscometer, volumetric flasks, beaker, test tubes, distiller, balance, rotary

evaporator, dissector, FTIR ,GC-MS, muffle furnace, kejaldhal, sieve, density bottle, PH meter, miller, 90 mm petri dish, test tubes, test tube holder, spectrophotometer, syringe, pipette, 37^0c incubator, 45^0c incubator, Biohazard, shaker, 8mm hole cylinder, refrigerators, small bottles, 121^0c autoclave, stove, balance, sterilizer and ruler.

3.2. Methods

3.2.1. Raw material preparation and proximate analysis of pumpkin seed

3.2.1.1. Raw material preparation

Pumpkin fruit was purchased one varieties (cucurbita pepo) from piassa market which is found in Addis Ababa, Ethiopia. Pumpkin fruit was undergo various processing in the course of its preparation for extraction. Pumpkin fruit shells were carefully cut open to expose the seeds which were embedded in an orange –yellow fibrous material. The seeds were removed from the fruit and thoroughly washed with distilled water to remove other component of pumpkin and impurities. After that Pumpkin seeds was cleaned manually in order to remove foreign material and impurities and then dulled manually to remove the outer seed coat and subsequently dried in an electric oven at 50^0C until a constant weight was obtained. Figure 3.1 shows Matured pumpkin(A), half pumpkin(B), hulled pumpkin seed(C), peeled pumpkin seed(D) sieve analysis(E) and sieved pumpkin seed meal(F) respectively.

3.2.1.2. Proximate analysis of pumpkin seed

Pumpkin seed chemical composition was determined by Association of Official Analytical Chemists (AOAC, 2005) methods, it was include:-

Moisture content

The moisture content was determined by drying the seeds in an oven at 105 ± 1°C to a constant weight using equation 3.1.

Moisture content(%) of the Pumpkinseed= $\frac{W1-W2}{W1} \times 100$ (3.1)

Where W_1= original weight of the sample before drying

W_2= weight of the sample after drying

Total ash

Ash was estimated by direct incineration of sample; igniting it in a Muffle Furnace (MF-1/02,PCSIR, Pakistan) at 550°C till grayish white residue, (AACC, 2000; Method No. 08-01).

Crude protein

Crude protein content was determined by using Kjeldhal Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany) as described in AACC (2000) Method No. 46-30.

Crude fat

Crude fat content was determined using hexane as a solvent in Soxtec System (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) according to the procedure give in AACC (2000) Method No. 30-25.

Crude fiber

Crude fiber was estimated in fat free samples by treating with 1.25% H2SO4; left over material was subjected to further treatment with 1.25% NaOH solutions. Crude fiber of the samples was determined through Labconco Fibertech, (Labconco Corporation Kansas, USA) as per procedure in AACC (2000) Method No. 32-10.

Carbohydrate (CBH)

CBH was calculated according to equation 3.2:

CBH % =100– (Moisture contents % + crude protein % + crude fat % + crude fiber % + ash %) (3.2)

The moisture content was expressed in g/100 g sample and the other values will be reported on dry basis. All the analyses will be performed in triplicate.

3.2.2. Size reduction and sieve analysis

After the moisture was removed by placing in an oven at 50^0C for 24 hours, the dried pumpkin seed was milled in Cross Beater Miller with a size of 1.8 mm and then the sample was shaken

using vibrating shaker for 8 minutes with amplitude of 10 mm. The sieve size was arranged in descending order of mesh size 3 mm, 1 mm and 0.2 mm to obtain particular size of 2 mm, 1.125 and 0.25 mm. This is due to investigate the effect of particles size on yield and quantity of oil (blame, 2006).

A B C

D E F

Figure: 3.1. Matured pumpkin(A), half pumpkin(B), hulled pumpkin seed(C), peeled pumpkin seed(D) sieve analysis(E) and sieved pumpkin seed meal(F) respectively

3.3. Soxhlet Extraction

3.3.1. Extraction process

Experimental work will be conducted using soxhlet equipment by solvent extraction process. The solvent used during extraction was ethanol. The result from soxhlet extraction like extraction time, particle size and pumpkin seed meal to solvent ratio was used as the starting parameter:

Initially the raw material pumpkin seed was prepared and cleaned well and then dried in order to remove the moisture contents. After the moisture is removed the pumpkinseeds was grinded and sieve in order to get good surface area or particle size and dried well again in order to get high yield. The grounded pumpkin seed samples were placed in a filter paper. The filter paper was then placed in an extraction chamber which is being suspended above a flask containing the solvent and below a condenser. Heat is being applied to the flask and the solvent evaporates and moves to the condenser where it is converted into liquid that trickles in to the extraction chamber containing the sample. The extraction chamber was made in such a way that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down in to the boiling flask. Finally, the oil and solvent was separated through rotary evaporator (simple distillation) at the end of the extraction process. Figure: 3.2. Shows Process description of extraction process of pumpkin seed essential oil

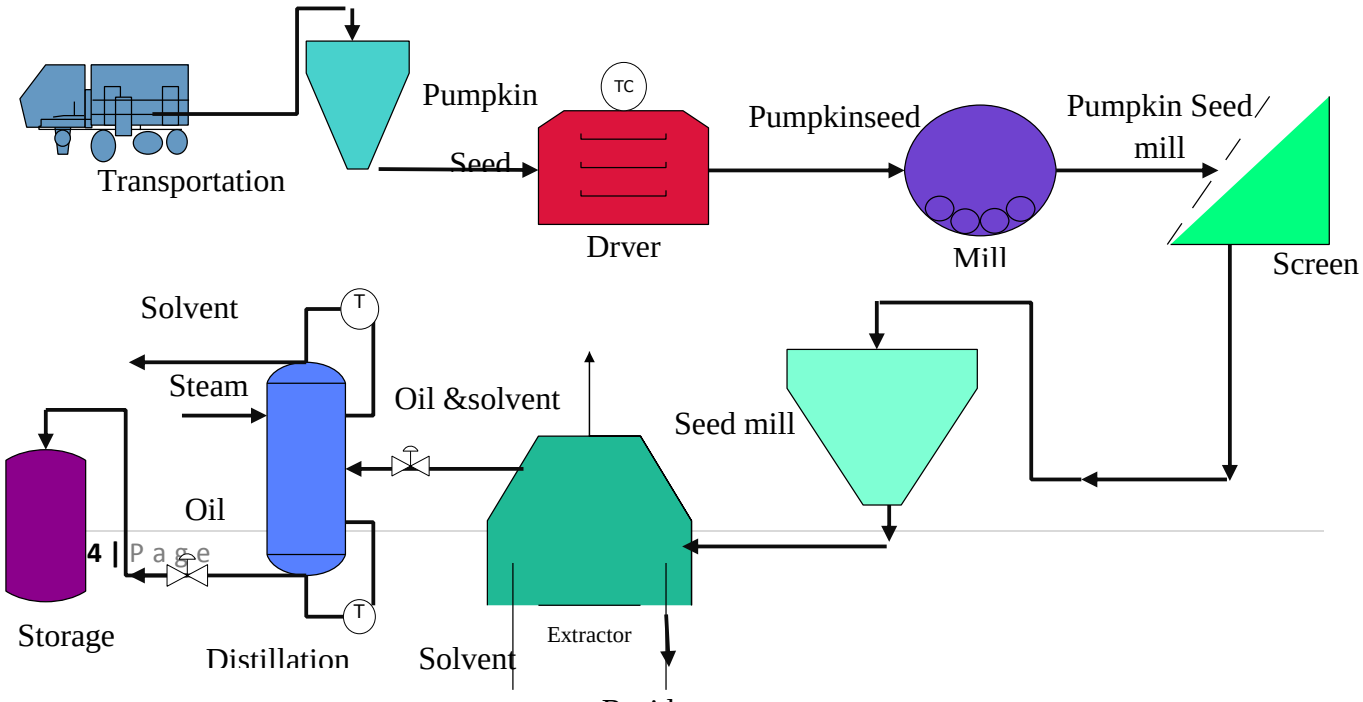


Figure: 3.2. Process description of extraction process of pumpkin seed essential oil

As explained above ethanol was poured into round bottom flask and 10g sample of seed meal was added to filter paper Extraction process variables was included extraction time: (2 to 6 hrs.), particle size (0.25 to 2 mm) and solvent to pumpkin seed meal ratio (1:10 to 1:20 w/v) under constant temperature (80^{o}c) on the yield and purity of essential oil will be performed. Solvent was recovered by rotary evaporator and residual solvent was removed by drying in an oven at 78°C for 1 h. Figure: 3.3 shows soxhlet extraction set up.

Figure: 3.3. Soxhlet extraction set up, ethanol and oil from soxhlet and extracted oil respectively

Determination of the yield of pumpkin seed oil extracted

10g (W_1) of the sample was placed in the thimble and sample was weight after extraction and dried in the oven. The yield was repeated for each run.

$$\% \text{ oil yield} = \frac{w_1 - w_2}{w_1} \times 100 \qquad (3.1)$$

Where: W_1=Sample was weighted before extraction and, W_2= sample was weight after extraction and dried in the oven.

3.4. Characterization of physicochemical analysis of extracted pumpkin seed essential oil

3.4.1. Determination of Moisture and volatile matter of oil

5 gm of oil was weighted and putted in a dish and then was dried in an oven at 105^0C for 1 hour. The dish was removed from the oven and cooled in a dissector and weighed. The process was repeated until a constant weight was observed and the moisture and volatile matter of the oil was determined in equation 3.2.

$$\text{Moisture \& volatile matter of essential oil} = \frac{w_1}{w_0} \times 100\% \quad (3.2)$$

Where W_1= loss in gram of the material on drying

W_0= weight in gram of oil taken for the test

3.4.2. Determination of specific gravity

Specific gravity of oil was determined using density bottle method. A washed, dried and weighed density bottle was filled with the oil sample and placed in a water bath for a temperature of 25 °C to be attainable. The weight of the oil was recorded after which the bottle was emptied, washed and dried again before refilling with water maintained at a temperature of 25 °C. The weight of water at this temperature was recorded and the specific gravity was calculated from the formula (A.O.A.C Official Method 920.212, 2000).

$$\text{Specific gravity} = \frac{(\text{Weight of the sample in the density bottle})}{(\text{Weight of equal volume of water})} \quad (3.3)$$

3.4.3. Determination of kinematic viscosity of oil

A kinematic viscosity of pumpkin seed oil was measured indirectly using Viscometer model. Initially, a sample was heated at a temperature of 30^0C. A sample of 35 ml oil was measured and fed to a sample holder of the Vibrio Viscometer. A sensor of the viscometer was immersed the oil and the dynamic Viscosity of oil was displayed on the Vibrio Viscometer screen at a temperature of 30^0C. Then the Kinematic Viscosity was calculated in equation 3.4.

$$\text{Kinematic viscosity of the oil (V)} = \frac{\mu}{\rho} \quad (3.4)$$

μ = Dynamic Viscosity ρ = Density of oil

3.4.4. **Determination of pH**

2 ml of the pumpkin seed oil was taken and placed in a clean dry 25 ml beaker and 13 ml of hot distilled water was added to the sample in the beaker and stirred slowly. Then it was cooled in a cold water bath to 25^0C. The pH electrode was standardized with a buffer solution first and then the electrode immersed in to the sample and the pH was read and recorded, (A.O.A.C Official Method of Analysis 960.19, 2000).

3.4.5. **Refractive index**

The refractive index of a substance is the ratio of the speed of light in a vacuum to the speed of light in the substance. For practical measurements, including this method, the scales of standard instruments indicate refractive indices with respect to air rather than vacuum. Refractive Index of the sample was determined indirect method using spectrometer (He-Ne Leather). It was ascertained that the temperature of the spectrometer is 25^0c at room temperature, wave number 632.8nm and the prism was clean and completely dry. The prism with sample was placed in the cuvet and adjusted the instrument and light to obtain the most distinct reading and then determined the refractive index using Brewster's angle. Brewster's angle formula was used to calculate refractive indices of the interface.

$$N = \tan \Theta b$$

Where: N = Refractive index, Θb =Angle of refraction, the refractive index of air is 1.

The index of refraction of oils is characteristic within certain limits for each kind of oil. It is related to the degree of saturation particularly to the extent of conjugation, but it is affected by other factors such as free fatty acid content, oxidation, and heat treatment

3.5. **Characterization of the chemical property of oil**

3.5.1. **Determination of saponification value**

2g of the oil will be placed in a conical flask to which 25ml of ethanoic potassium hydroxide (0.1M) will be added and the mixture allowed boiling gently for about 1hr. With shaking, at regular intervals of 5 min. Few drops of phenolphthalein indicator, as specified by International Standards Organization (ISO 3657, 1988) will be added to the warm solution and then titrated with 0.5M HCl. The end point will be reached when the pink colour of the indicator just disappeared. The same procedure will be followed for the blank. The saponification value (SV) is given by:

$$SV=56.1 \times \frac{N(V_0-V_1\ )}{m} \quad (3.5)$$

Where: V_O = volume of HCl solution used for the blank test, V_I = volume of HCl solution for the determination, N = actual molarity of HCl used, and m = mass of sample

3.5.2. Determination of acid value

25ml of Toluene and 25ml of ethanol Will be mixed in a 250ml beaker. The resulting mixture will be added to 2g of oil in a 250ml conical flask and few drops of phenolphthalein will be added to the mixture. The mixture will be titrated with 0.1M KOH to the end point with consistent shaking for which a dark pink color will be observed and the volume of 0.1M KOH (V_0) Will be noted, (A.O.A.C, 2000).

$$\text{Acid value (AV)}=56.11 \times \frac{V \times C}{m} \quad (3.6)$$

Where V =Volume of potassium hydroxide (ml), C=Concentration of potassium hydroxide, 56.11 =Molecular weight of potassium hydroxide, m= sample weight

3.5.3. Determination of Iodine value

The amount of iodine consumed is determined by Titrating the iodine released (after adding KI) with a standard Thiosulphate. Procedure: 0.3 g of fats was weighed in to a small weighing dish and placed in a 250 cm^3 conical flask 10 cm^3 of carbon tetra chloride was added to the samples. To the entire flask an equal quantity of about 25cm^3 wigits reagents was added using a burette, this was mixed well and kept in the dark for an hour, after that it was titrated with standard 0.1M sodium thiosulphate solution while adding 15cm^3 of 10 % potassium iodide solution and 100 cm^3 of distilled water using starch as an indicator. The iodine value (I.V) is given by the expression (A.O.A.C Official Method 993.20):

Where: C = Concentration of sodium thiosulphate used, V_1= Volume of sodium thiosulphate used for blank, V_2= Volume of sodium thiosulphate used for determination, M = Mass of the sample

$$\text{Iodine value(IV)}=12.69 \times \frac{C(V_1-V_2)}{M} \quad (3.7)$$

3.5.4. Fourier Transform Infrared ray (FTIR)

The FT-IR spectrum of the pumpkin seed essential oil was obtained using Perkins Elmer Spectrum 65 FT-IR spectrometer in Addis Ababa University and functional groups was determined with the help of IR correlation charts. The IR spectrum was reported in % transmittance. The wave number region for the analysis was 4000-400 cm-1(in the mid-infrared range) (Nurrulhidayah et al., 2011).

3.5.5. Gas chromatography-mass spectroscopy

The component identification was achieved by the GC-MS analysis using HP 5890 series GC equipped with mass selective detector (MSD), HP 5972 series (German) in LIDI, Addis Ababa, Ethiopia. Helium was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1µl will be employed, injector temperature 250^{O}C and Ion-source temperature 280^{O}C. The oven temperature was programmed from 50 (isothermal for 4min.), with an increase of 3 /min, to 280^{O}C and held for 10 min. isothermal at 280^{O}C .Total GC running time was 90.67 min. The components of essential oil were identified on the basis of comparison of their retention time and mass spectra with published data (Analytical Methods Committee, 1984; Joinery. Y and RP, 1976) and computer library matching or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds.

3.6. Experimental design

The experimental design follows a general factorial design; factorial designs are the most efficient for experiments that involve two or more factors. By factorial design in each complete trial or replication of the experiment all possible combinations of the levels was investigated. The analysis of variance (ANOVA) was used as one of the primary tools for statistical data analysis using a design Expert software 6.0.8, (Montgomery d,. 2002). Factors and respective ranges of the experiments are described in Table 3.1 blew.

Table: 3.1. Factors and respective ranges of the experiments

Factor	Name	Units	Low actual	High actual
A	Particle size	mm	0.25	2
B	Extraction time	hour	2	6
C	Meal:Solvent ratio	g/ml	0.05	0.1

Number of runs in 2 level full factorial with 3 factors, 3 repliction, 6 center points, and 1 block

$$=3 *2^3+6=30$$

30 experiments was conducted, one pumpkin variety, two reaction times, two particle size and two meal to solvent ratio. 10g of pumpkin seed meal was used for each treatment replications. The experiment was conducted in split plot with three replications and six center points.

3.7. Evaluation of extracted essential oil for antimicrobial activity

3.7.1. Antimicrobial disc and agar preparation

Discs of about 6mm diameter were made from What man's No.1 filter paper using a paper puncher. The discs were transferred in to Bijou bottles and sterilized in the oven at 121^{o}C for 15 minutes. Sensitivity discs were prepared by serial doubling dilution of the extract in Dimethyl Sulfoxide (DMSO). Figure: 3.4. Shows as set up of antimicrobial activity sterilized petir dish and syringes (A) , Mueller Hinton agar on balance(B), sterilized Mueller Hinton agar (C) sterilized Mueller Hinton broth and bacteria slant with their colonies(D) serial dilution of pumpkin seed oil in DMSO and diluted pumpkin seed oil on activity(F) respectively.

3.7.2. Microbial strains

The essential oils of pumpkin seed (cucurbita pepo) was tested against a panel of microorganisms including Staphylococcus aureus and Escherichia coli. The microorganisms were supplied from Ethiopian public health institute which is found in Addiss Ababa, Ethiopia.

3.7.3. Preparation of stock solutions

Pumpkin seed essential oil can be dissolved in solvent such as ethanol, toluene and DMSO. Among those solvent DMSO has good to dissolve the pumpkin seed essential oil, So that it is better to used DMSO as solvent. The stock solutions of DMSO and the essential oil were made by dissolving 1ml of the essential oils in 1ml of DMSO. From the stock solutions serial dilutions were made to obtain the test solutions of concentration ½, 1/4, 1/8 and 1/16 (Del, et al., 2009).

3.7.4. Screening for antimicrobial activity

The antimicrobial activity was tested through disc diffusion method. Mueller Hinton agar was used as the standard test medium for bacteria. Fresh cultures was prepared and used to inoculate 50 mL of Mueller Hinton broth that was incubated at 35 ºC for 18 h. Few colonies of the confirmed isolates were dispensed in sterile normal saline and match turbidity in the 0.5 McFarland Standard (approximately 10 8 CFU mL-1) Suspensions of microorganisms were incorporated in the appropriate medium (1 ml/100 ml media)for sensitivity test as described by NCCLS(1999).

The agar plates was prepared in 90 mm Petri dishes with 22 mL of agar medium giving a final depth of 3 mm. Cylinders (diameter 5.5 mm) was placed on the inoculated agar surfaces and filled with 100 µL of diluted oil in DMSO. Each 100 µL were having 6.25, 12.5, 25 and 50% v/v (ml/ml) of essential oil. For each microbial species, negative control was maintained where 100µl of DMSO alone without the drug was used. Also, conventional drugs were used for positive controls but in this case we don't have positive control. In the central hole of the different Petri dishes, the control was put for each organism. All plates were aerobically incubated at 35 ºC for 18-24 h (Del, et al., 2009).

The results were recorded by measuring the diameter of the zones of growth inhibition surrounding the wells (cylinders). The net effect of the pumpkin seed oil was obtained by subtracting the diameter of the zone of inhibition due DMSO alone from the diameter of the zones of inhibition due to the pumpkin seed oil diluted in DMSO. Each test was performed in triplicate and the results was shown as means (Del-vechio-vieira, et al., 2009).



A B C

D E F

Figure: 3.4. Sterilized petir dish and syringes (A) , Mueller Hinton agar on balance(B), sterilized molar Hinton agar (C) sterilized Mueller Hinton broth and bacteria slant with their colonies(D) serial dilution of pumpkin seed oil in DMSO and diluted pumpkin seed oil on activity(F) respectively.

3.7.5. Determination of the minimum inhibitory concentration

The minimal inhibitory concentration (MIC) was defined as the lowest concentration of oil or active compound inhibiting the visible growth of bacteria. Control samples (positive and negative) were incubated under the same conditions. It was determined through agar dilution method. Bacteria were grown in Mueller Hinton broth (brain heart infusion liquid medium) for 6 h. After this, Few colonies of the confirmed isolates were dispensed in sterile normal saline and match turbidity in the 0.08-0.1 McFarland Standard (approximately 10 8 CFU mL-1) using spectrophotometer, suspensions of microorganisms were incorporated in the appropriate medium (1 ml/100 ml media)for sensitivity test as described by NCCLS (1999). 20 µL was inoculated in tubes with Mueller Hinton broth supplemented with four different serial dilution (1/16, 1/8, 1/4 and 1/2) of the oils . After 24 h at 37 ºC, the MIC of each sample was measured through optical density in the spectrophotometer (620 nm), through the comparison of the sample readout with the non-inoculated Mueller Hinton broth. All determinations was performed in triplicate (Del-vechio-vieira, et al., 2009)

4. RESULT AND DISCUSSION

4.1. Proximate analysis

Table: 4.1 General proximate analyses of pumpkin seed

Parameter tested and result (%)					
Crude protein	Crude fiber	Moisture	Crude fat	Ash	Carbohydrate
22.5±0.31	7±0.09	5.21±0.12	50±0.21	4.5±0.19	10.37+0.079

Proximate composition is important in determining the quality of raw materials. Pumpkin seeds were analyzed for different quality attributes; contains 5.21 ± 0.12% ,22.5± 0.31%, 50.5± 0.112%, 7±0.09%, 4.5± 0.19% and 10.37±0.079% of moisture, crude proteins, crude fat, crude fiber, ash content, carbohydrate respectively shown in Table:4.2.

The moisture content of the five pumpkin seed samples having mean mass gives 5.21 ±0.12. When seed is delivered to the mills in quantities greater than the daily capacity of the plant, it is customary to store it in seed-houses or silos for future use. So that the moisture content of the

seed must be reduced in order to minimize the deterioration and have long shelf life. Shelf life is inversely proportional to the moisture. As less as moisture content is, shelf life will be more. The result obtained was agreed with those reported in literature (Gohari et al., 2011 ; Hamed, et al., 2008 ; Milani et al., 2007 ; Nwofia, et al., 2012).

Findings of present research regarding characterization were in close conformity with the values described in the literature (Kwiri et al., 2014; Milani et al., 2007; Petkova & Antova, 2015; Popa et al., 2010; Srbinoska, et al., 2012) slight differences in pumpkin seed proximate composition such as ash and carbohydrate contain are the result of seed characteristics, genus, species, harvest conditions, degree of fruit maturation, environmental factors like climate and cultivation zone characteristics might be a possible reason for these variations. Moreover, difference in genetic makeup could also be a contributing factor as indigenous variety was tested in the trial. The composition of pumpkin seeds and moisture, fat, protein, ash and total carbohydrates contents reported in literature were in the range of 3.8-7.0, 35-60, 20.85-31.2, 2-10 and 20.0-35% respectively (Kwiri et al., 2014; Rodríguez-miranda et al., 2014). Also, the oil content of the pumpkin seed in the present study was found to exceed, or be comparable to, that of some common edible oils such as cottonseed (22-24%), safflower (30-35%), soybean (18-22%), rapeseed (40-48%), and olive (12-50%) (Gohari etal., 2011). Therefore, the pumpkin seed can be considered as a potential source of vegetable oil for domestic and industrial purposes. In addition, the protein content of the pumpkin seed from this study was higher than those of other oilseeds, e.g. cottonseed (21.9%), and sesame (18.7%), and that of animal proteins (16.0-18.0%) such as lamb, fish, and beef (Gohari et al., 2011). Pumpkin seeds are also considered to be rich in protein.

4.2. Oil extraction

Table: 4.2 Percentage oil yields of pumpkin seed

Std	Run	Block	A	B	C	%Yield
1	26	Block 1	0.25	2	0.05	74
2	23	Block 1	0.25	2	0.05	75
3	14	Block 1	0.25	2	0.05	72
4	1	Block 1	2	2	0.05	64

5	9	Block 1	2	2	0.05	66
6	18	Block 1	2	2	0.05	65
7	20	Block 1	0.25	6	0.05	94
8	5	Block 1	0.25	6	0.05	92
9	7	Block 1	0.25	6	0.05	93
10	12	Block 1	2	6	0.05	82
11	4	Block 1	2	6	0.05	84
12	28	Block 1	2	6	0.05	86
13	15	Block 1	0.25	2	0.1	68
14	29	Block 1	0.25	2	0.1	66
15	6	Block 1	0.25	2	0.1	68
16	17	Block 1	2	2	0.1	60
17	21	Block 1	2	2	0.1	62
18	25	Block 1	2	2	0.1	63
19	22	Block 1	0.25	6	0.1	88
20	27	Block 1	0.25	6	0.1	86
21	30	Block 1	0.25	6	0.1	84
22	16	Block 1	2	6	0.1	78
23	2	Block 1	2	6	0.1	79
24	8	Block 1	2	6	0.1	80
25	24	Block 1	1.13	4	0.08	88
26	3	Block 1	1.13	4	0.08	90
27	10	Block 1	1.13	4	0.08	90
28	11	Block 1	1.13	4	0.08	92
29	13	Block 1	1.13	4	0.08	88
30	19	Block 1	1.13	4	0.08	90

From Table 4.2 the maximum percentage oil yield obtained from pumpkin seed was 94% at average particle size 0.25 mm, extraction time of 6 hour and meal to solvent ratio of 0.05g/ml.

Whereas the minimum percentage oil yield from pumpkin seed was 74% obtained at average particle size of 2 mm, extraction time 2 hour and meal to solvent ratio 0.1 mg/ml.

A percentage extraction yield of 94 was closer to the result reported by (patel, 2013; Widy-tyszkiewicz & Widy-tyszkiewicz, 2013) using ethanol as a solvent with extraction time of 6 hour. Leaflets, 2013, reported as yield of 90% using hexane as a solvent and with extraction time of 5 to 6 hour. Liauw et al., (2008), also reported that the oil yield of neem seed using ethanol was 89% at 50°C and 0.425-0.71mm particle size.

The following table: 4.4. shows analysis of variance (ANOVA) obtained from design expert software, which tells as the significance of different factors.

4.3. Physiochemical characterization of extracted oil

Using process parameters that gave a maximum oil yield (average particle size 0.25mm, extraction time of 6 hour and pumpkin seed meal to solvent ratio of 0.05g/ml) extracted was studied for its physical and chemical properties.

4.3.1. Moisture and volatile matter of oil

Table: 4.3. Moisture and volatile matters of pumpkin seed oil

Time in hours	Wt.at time 0hrs	Wt.at time=1hrs	Wt.at time 2hrs	Weight in gm. loss by (2-0) hrs.
Weight in gm.	5	4.93	4.93	0.07 $\pm$ 0.21

Moisture and volatile matter of oil = $\frac{0.07}{5} \times 100\% = 1.4\%$

4.3.2. Specific gravity

Specific gravity of pumpkin seed oil=$\left(\frac{71.96-24.416}{76.56-24.416}\right)=0.91178$

Hence the density of oil can be determined using:

$$SG = \frac{\rho \text{ oil}}{\rho \text{ water}}$$

ρ oil of seed oil = SG* ρ water = 0.91178*1000 kg/m^3

Therefore density of pumpkin seed oil was 911.78 kg/m^3

4.3.3. Kinematic viscosity

Substituting the dynamic viscosity of pumpkin seed oil = 3.2mpa.sec = 3.2 *10^6kg/ m.s and

Density of pumpkin seed oil = 911.78kg/m^3

$$\text{Kinematic viscosity (V)} = \frac{\mu}{\rho}$$

$$\text{Kinematic viscosity pumpkin seed oil} = \frac{\text{dynamic viscosity}}{\text{density oil}}$$

$$= \frac{3.2 * 106 \text{kg/m.s}}{911.78 \text{kg/m3}} = 35.09 \text{m}^2/\text{s}$$

4.3.4. pH value

Therefore the pH value of pumpkin seed oil was slightly acidic which 5.246 + 0.01 is. In preparation of antimicrobial activity, skin and hair care materials, the preferable pH value is in the range of 3.5-6.5, (Mueller et al., 2000). The obtained pH value of pumpkin seed oil is in the range to be used in for antimicrobial activity.

4.3.5. Refractive index

It was found that Pumpkin seed essential oil has the Brewster's angle equal to 55.751. Put these values in to equation (3.7) and calculate refractive index, N.

$N_1 = \tan(\Theta_b) = \tan(55.751) = 1.4687$

4.3.6. Saponification value

The saponification value of the pumpkinseed oils and were found to be 189.8 $\pm$ 0.31. These shows that more alkali would be required to enable it neutralize the available free fatty acid

liberated by the oil. The saponification values of pumpkin seed oils are highly comparable with other oil seed saponification value: palm kernel, breadfruit, groundnut, coconut, soybean, and dacryodes to be in the range of 195 to 261 (mg KOH/g sample). Eze, (2012) reported that SV for palm oil is 200 (mg KOH/g sample), for groundnut is 193 (mg KOH/g sample) and for coconut oil is 257 (mg KOH/g sample).

Saponification value of pumpkin seed oil was not affected by variety; the saponification value of cucurbita pepo was ($p<0.5$) higher than that of c. maxima by 1.63%. Similarly, the saponification value in this study was lower when compared to the saponification value of cocosnucifera oil (246 mg KOH/g oil), cocoa (188 – 195 mg KOH/g oil), but higher in comparison to the saponification value of rubber seed oil (13.46 mg KOH/g oil), annona oil (100.84 mg KOH/g oil). Nevertheless, it was similar with the pumpkin seed oil reported and melon seed oil (184.4 mg KOH/g oil). The result also suggest that pumpkin seed essential oil is more suitable for used for production of liquid soap, cosmetics, shampoos and creams (Bwade, et al., 2013).

4.3.7. Acid value

Acid value represents free fatty acid content was 1.23± 0.61 and 0.62± 0.23 this is due to enzymatic activity, and is usually indicative of spoilage. Its maximum acceptable level is 4 mg KOH/g oil (CODEX Alimentarius Commission, 1982), for recommended international standards for edible Arachis oil, (Eze, 2012). From Table 4.11 properties of pumpkin seed oil the average acid value of pumpkin seed oil of 1.23 which is relatively smaller. The acid value of oil from c. pepo was ($p<0.5$) difference with the acid value of pumpkin seed oil 1.99 mg KOH/g oil reported by Moo-huchin et al., 2013. It was lower in comparison to the acid value of Palm kernel oil (19.035 mg KOH/g oil), Groundnut seed oil (8.976 mg NaOH/g oil), Coconut seed oil (3.927 mg NaOH/goil), soybean (2.85 mg NaOH/g oil) and rubber seed oil (8.17 mg KOH/g oil) which is reported by Eze, (2012).

Eze, (2012) reported that the acid value obtained from this research can be used to check the level of oxidative deterioration of the oil by enzymatic or chemical oxidation. The acid value is expected to range from 0.00 to 3.00 mg KOH/g oil before it can find application in cooking. The low acidity of oil is an indication of oil which is free from hydrolytic rancidity and enables the direct use of such oil without further neutralization. Therefore the result obtained indicated that pumpkin seed oil can used directly without further neutralization. The low free fatty acids

content (0.3 and 0.26) was indicative of low enzymatic hydrolysis. This can be an advantageous that pumpkin seed oil cannot develop off (rancidity) flavor during storage.

4.3.8. Iodine value

The iodine value is a characteristic of the unsaturation of fatty acids or its esters and the amount of iodine (in gram) necessary to saturate 100 g of oil sample. Lipids with unsaturated fatty acids (containing one or more double bonds) are easily assimilated and broken down to produce calorific energy than saturated fatty acids. The higher the iodine value, the more unsaturated the oil, the longer shelf life and higher quality. However, when the iodine value becomes too high, the stability of the oil reduces because it is more likely to undergo oxidation (Marie et al., 2015).

Testing of iodine value of pumpkin seed oil fat has been conducted at Ethiopian public health institute and Analytical testing service laboratory and it was found to be 97.5 gm/100gm. The result indicated that pumpkin seed oil has high iodine value, which indicates high resistance to oxidation and longer shelf life.

The result obtained for the Iodine value for the pumpkin seed oils was higher values, increase in the average degree of un-saturation of the oil, as such, the amount of iodine which can be absorbed by unsaturated acids would be higher. As a result of their agreement with standard pumpkin seed oil could be classified as non-drying oils; since their iodine values are lower than 100 (gI2/100 g sample) and it could be used extensively as lubricants and hydraulic brake fluids. The iodine values obtained here are comparable to the literature value of castor oils and olive oils, both of which are non-drying oils (Marie et al., 2015).

Table: 4.4. Physical property of pumpkin seed oil

Physical parameter	**Value**
Color	Dark - brown
Refractive index	1.464
Specific gravity (g/cm^3**)**	0.91178
PH	5. 26$\pm$ 0.01
Kinematic viscosity (m^2/s)	35

Density (kg/m^3)	911.78
Moisture and volatile component	1.4%

Table: 4.5. Chemical parameter of pumpkin seed oil

Chemical parameter	**Value**
Free fatty acid	0.62 ±0.23
Acid value(mg KOH/g)	1.23± 0.61
Saponification value(mg KOH/g)	189.8±0.1
Iodine value (I g/100g oil)	97.45

Pumpkin seed oil extracted through solvent extraction was tested for various physical & chemical characteristics and fatty acid profile (Table: 4.4 and Table: 4.5). Means values for physical parameters of essential oil including specific gravity, pH, kinematic viscosity, density moisture and volatile and refractive index values were: 0.91178, 5.26±0.01, 35, 911.78, 0.07 and 1.468 respectively. Likewise, means for chemical parameters like free fatty acid, iodine, acid value and saponification value were 0.62 ±0.23, 97.5 I_2g/100g oil, 1.23± 0.61mg KOH/g oil and 189.8±0.1mg KOH/g oil respectively non-significant difference with literature reported by Poiana et al., (2002).

Physical parameters like specific gravity, refractive index, are important in quantitative estimation of fat and oils and values observed in the present research remained in the ranges described in literature, (Leaflets, 2013; Sito et al., 2005; Srbinoska et al., 2012; Tsaknis & Lazos, 1997)

Chemical attribute like iodine value indicates the presence of unsaturated fatty acids and higher value is an indication of the presence of lower amount of saturated fats and vice versa. The iodine value usually ranged from 15.0 to 150mg/100g in vegetable oils. The present findings are in corroboration with the values reported earlier by Nábrádi, et al., (2014) and Bikash & Bora,(2018).

Results of physical and chemicals characteristics were in line with studies (Nábrádi, et al., 2014; Bikash, et al., 2018). Moreover, results of fatty acid profile provide evidence of its rich nutritional profile in terms of polyunsaturated fatty acids.

4.4. Determination of the functional groups present using FT-IR

Infra-red (IR) spectrum was recorded on a Perkin-Elmer FT-IR spectrometer. About 1 mg of the isolated compound was prepared as KBr pellets and employed for recording the IR spectrum (frequencies between 4000 and 400 cm-1) Nurrulhidayah, et al., (2011).

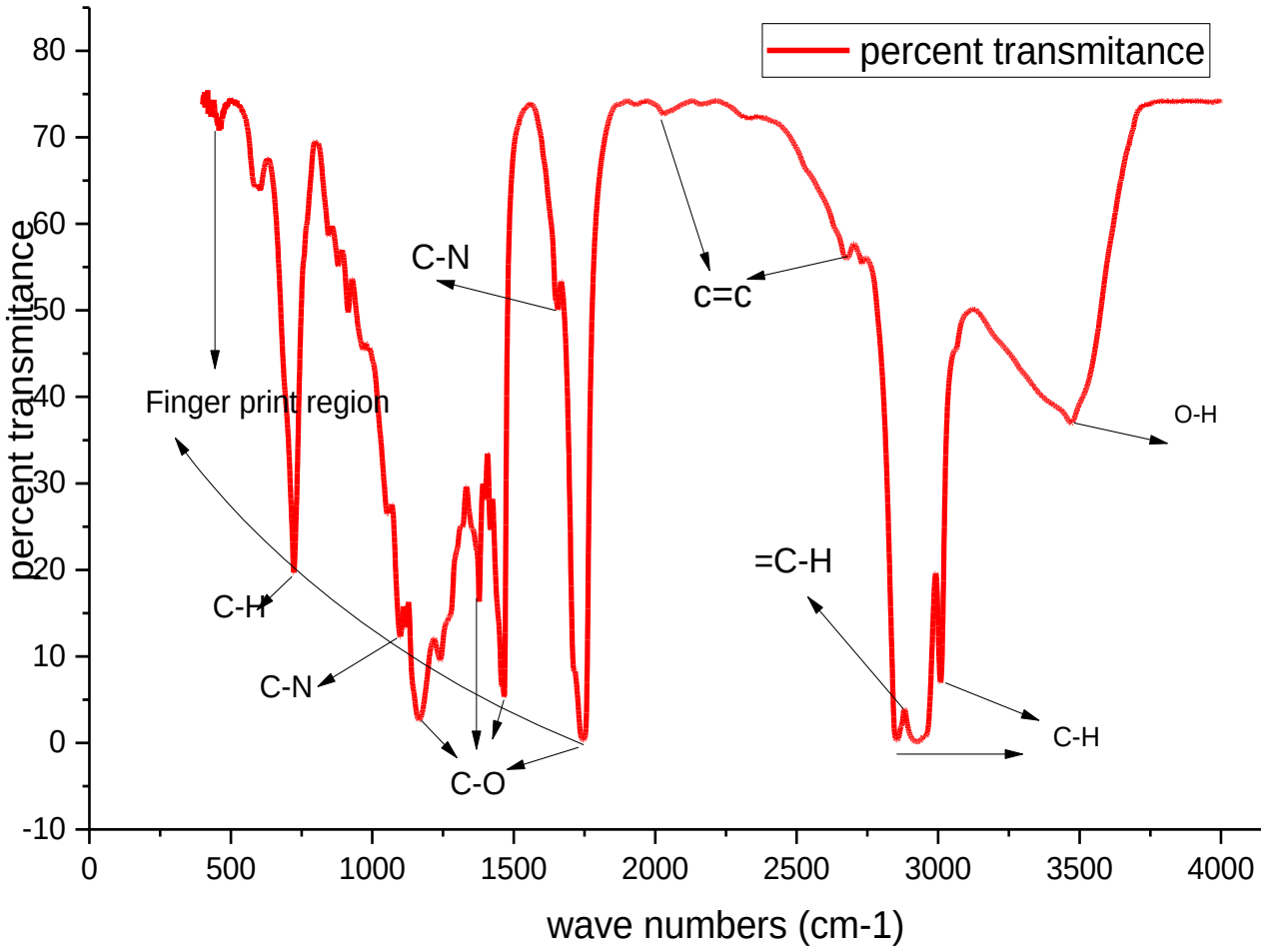


Figure: 4.1. Pumpkin seed oil functional group

Figure: 4.1 shows the functional groups present in the pumpkin seed oil. The functional groups present in the pumpkin seed oil were determined by comparing the vibration frequencies in wave numbers of the sample spectrograph obtained from an FT-IR spectrophotometer with

those of an IR correlation chart. FTIR spectra of pumpkin seed oils appear fairly similar. In the FT-IR spectrum of pumpkin seed essential oil the absorption band or frequency from 3488cm^{-1} and 3056cm^{-1} showed the presence of medium indicate is the region from 3200-3600 of functional groups such as strong broad stretch free vibration, H-bonded, C-H stretch for presence of alkene, aromatic, alkane and stretching of methyl ester. The stretch band at 3056 cm^{-1} =C-O and C-H revealed the presence of alkene and aromatic. 2989cm^{-1} and 2848cm^{-1} The range of wave number from 2850-3000cm^{-1} indicate the presence stretch vibration C-H alkane and -C-H bending vibration. The strong peak in the range of 2333.06 cm^{-1} indicate C=C double bond stretch vibrated with functional group alkene. esterified carboxyl group C=O, from 1700-1600cm^{-1} asymmetric carboxyl stretching and the peak is 1630.42cm^{-1} indicate C-N Aliphatic amine free carboxyl group compound. A medium-weak band between 1680-1600 cm^{-1} showed the presence of alkenes C=C stretch. A wave number 1751.07cm^{-1} indicates the existence of, C-O unsaturated esters and aliphatic amine functional groups. From the spectra of selected oils, the bands at 1445.85 and 1108.47 cm^{-1} are evident, which are correlated to stretching vibration of C-O ester, carbocyclic and alcohol groups. A strong absorption 1016.47cm^{-1} indicated the presence of C-N Aliphatic amines, carbohydrate groups. A strong absorption 625cm^{-1} indicated the presence of C-H Alkane carbohydrate, aromatic ring but from 1500-400 finger print. Nurrulhidayah, et al., (2011); reported that similar result for Nigella sativa L. seed oil.

4.5. Gas chromatography –Mass spectroscopy

Table: 4.6. Library lists of total components of Pumpkin seed oil & area (%)

NO	Retention time	Compound name	Matching	% of total

1	30.116	Phenol, 2,4-bis(1,1-dimethylethyl)	99	0.11
2	37.035	Methyl tetradecanoate	96	0.08
3	38.115	Tetradecanoic acid	98	0.17
4	42.978	Hexadecanoic acid, methyl ester,	99	8.72
5	42.978	pentadecanoic acid, methyl ester	94	8.72
6	44.017	n- hexadecanoic acid,	99	1.55
7	44.017	tetradecanoic acid	93	1.55
8	44.814	Hexadecanoic acid, ethyl ester,	98	1.99
9	44.814	pentadecanoic acid, methyl ester	94	1.99
10	47.598	9,12- octadecadienoic acid (Z,Z), methyl ester	99	30.18
11	47.598	9,12- octadecadienoic acid, methyl ester	99	30.18
12	47.598	10,13- octadecadienoic acid, methyl ester	99	30.18
13	47.731	9- octadecadienoic acid, methyl ester(E),	99	13.41
14	47.731	9- octadecadienoic acid(Z), methyl ester	99	13.41

15	48.343	Methyl strearate	99	5.95
16	48.603	9,12- octadecadienoic acid (Z,Z), Ethanol	99	1.82
17	48.759	Oleic Acid, 9- octadecadienoic acid, (E)-	99	2.15
18	49.215	9,12- octadecadienoic acid, ethyl ester	99	6.25
19	49.360	Ethyl oleate, 9- octadecadienoic acid, ethyl ester	99	3.15
20	49.810	Methyl 10- trans, 12 cis-octadecadienoate,	99	0.11
21	50.012	octadecadienoic acid, ethyl ester,	99	1.37
22	50.012	Heptadecanoic acid ethyle ester	94	1.37
23	51.849	Cyclohexane ethanol, 4-methyl-beta-methylene	87	0.11
24	51.288	9- octadecadienoic acid, 12-hydroxy- methyl ester, [R—(Z)]	94	0.25
25	52.576	Tricosane, Nonadecane,2,6,10,14-tetramethyl-	97	0.28
26	53.223	Methyl 18-methanonadecanoate, Eicosanoic acid, methyl ester	99	0.27
26	56.469	9,12- octadecadienoic acid (Z,Z)-Bicyclo[10.1.0] tridec-1-ene,	98	3.51
27	56.469	8-Hexadecyne	86	3.51
28	57.133	Glycidol strearate	94	0.36
29	57.341	Hexadecenoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester,	91	6.5
30	57.786	Methyl 20-methyl-heneicosanoate,Docosanoic acid, methyl ester	98	0.11
31	58.144	Bis (2-ethylhexyl) phthalate, phthalic acid, di(2-propylpentyl)ester	98	1.47
32	60.437	3-(2,2-dideuterobutyl)- thiophene-1,1-dioxide	53	0.14
33	61.54	9,12- octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl),	93	5.21

		ethyl ester		
34	61.54	9,12- octadecadienoic acid (Z,Z)2,3dihydroxypropyl ester	83	5.21
35	61.117	octadecadienoic acid,2,3-dihydrioxypropyl ester	83	2.31

4.5.1. Fatty acid composition of pumpkin seed oil

Table: 4.7. Fatty acid composition of pumpkin seed oil

No	Retention time	Fatty acid	Library search Compound name	Matching Quality %	% of total
1	37.035	Myristic acid(14:0)	Methyl tetradecanoate	98	0.17
2	42.978	palmitic acid (16:0)	Hexadecanoic acid, methyl ester,	99	8.72
3	44.618	Linoleic acid(18:2)	9,12- octadecadienoic acid(z,z) , methyl ester	99	0.5
4	47.731	Oleic acid(18:1)	9- octadecadienoic acid, methyl ester(E), 9- octadecadienoic acid(Z)	99	13.41
5	47.598	Linoleic acid(18:2)	9,12- octadecadienoic acid (Z,Z), methyl ester	99	30.18
6	48.759	Oleic acid(18:1)	9- octadecadienoic acid, methyl ester(E),	99	2.15
7	49.215	Linoleic acid(18:2)	9,12- octadecadienoic acid, ethyl ester	99	6.25
8	56.469	Linoleic acid(18:2)	9,12- octadecadienoic acid (Z,Z)-Bicyclo[10.1.0] tridec-1-ene,	98	3.51
9	57.341	palmitic acid (16:0)	Hexadecenoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester,	91	6.5

10	61.54	Linoleic acid(18:2)	9,12- octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl), ethyl ester	93	5.21
11	62.117	Steric acid(18:0)	Octadecadienoic acid,2,3-dihydrioxypropyl ester	83	2.34

	Fatty acid composition %
Saturated fatty acid	17.8 22.44
Monounsaturated	15.89 20.7
Polyunsaturated	45.65 57.5
Total unsaturated fatty acid	61.54 78.5
% sat / %unsaturated	0.28

The fatty acids composition of optimum pumpkin seeds oil yield is presented in Table: 4.10 which can be used to evaluate its stability and nutritional quality. A higher degree of oil unsaturation makes it more susceptible to oxidative deterioration. On the other hand, there are considerable data to recommend a reduction in monounsaturated and a moderate increase in saturated and n-3 and n-6 polyunsaturated fatty acids in human nutrition in order to prevent coronary heart disease and other diseases. The composition of fatty acids varies depending on several factors including variety, growing area, climate and ripeness. The fatty acid composition is a major determinant of oil quality.

In this result, the saturated fatty acids (SFA) were: Palmitic acid (C16:0) and Steric acid (C18:0), the monounsaturated fatty acids (MUFA) include Oleic acid (C18:1) while the polyunsaturated fatty acid (PUFA) include Linoleic acid (C18:2), Eicosenoic acid (C20:1) and Myristic acid(C14:0) were found in the pumpkin seed oil and they constituted more than 97% of the total amount. This fatty acid profile is confirmed by several authors, (Gohari & Haddad, 2011) The pumpkin seed oil contained 22.4% saturated fatty acids, with the major one being palmitic acid (19.1%) followed by stearic acid (2.97%), while it was high in

unsaturated fatty acids with a total content of 77.7%. This total content of the unsaturated fatty acids was similar to that of the other studies on C. pepo and all pumpkin species in Cucurbitaceae, (Stevenson, et, all, 2007)

The main unsaturated fatty acids were linoleic acid followed by oleic acid with concentrations of 57.5 and 20.2 %, respectively. In most other investigations on the fatty acid composition of C. pepo the percentage of linoleic acid was higher (43.1-55.6%) than that of oleic acid (20.4-37.8%), while, in the present study, the percentages of linoleic and oleic acids were almost the same (57.5 and 20.2%, respectively). Also, the level of other fatty acids in the pumpkin seed oil was very low, similar to the results reported in the literature (Stevenson, 2007). This results were in agreement with other studies which, observed that, linoleic acid was the principal fatty acid followed by oleic acid in pumpkin seed oil.

It indicates that the fatty acid composition of pumpkin seed oil is quite close to that of melon seed oil. The presence of high amounts of the essential linoleic acid suggests that the pumpkin seed oil is highly nutritious. Orsavova & Vicha,(2015), reported that polyunsaturated fatty acids (PUFAs) have an impact on human health in the prevention of, particularly, cardiovascular disease (DVD), coronary heart disease and cancer; further, inflammatory, thrombotic and autoimmune disease; hypertension; diabetes type two, renal diseases; and rheumatoid arthritis, ulcerative colitis, and Crohn's disease. From GC-MS results pumpkin seed oil have also high percentage of polyunsaturated fatty acids; it can be used for the prevention of above mention diseases.

4.6. Experimental design

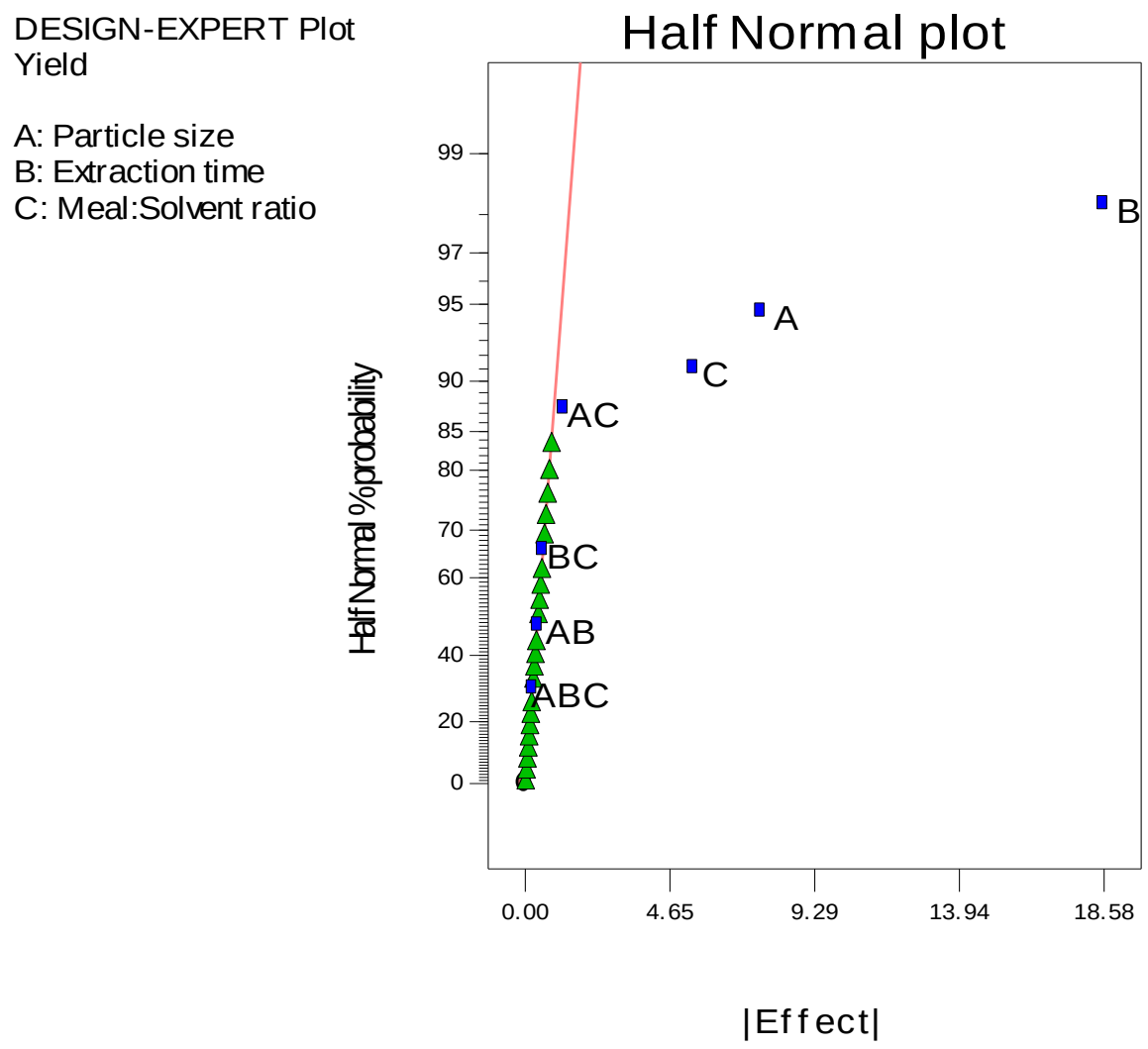


Figure: 4.2. Half Normal % probability plot virsus Effect (Factors)

From figure 4.2 it was implied that the effect each factor on the yield of pumpkin seed oil was shown. As it is seen from the figure, factor B, extraction time, has high studentized effect of 18.58 on the yield which accounts 59.21% percentage contribution, factor C, pumpkin seed meal to solvent ratio, has low effect compared to factor A and factor B, which has studentized effect of 2.71 which has a percentage contribution of 5.03% and factor A, average particle size, has more effect than factor C, pumpkin seed meal to solvent ratio but less effect than extraction time on the yield of pumpkin seed oil which has a studentized effect of 9.23 and percentage contribution 9.86%. The other important thing seen from the above figure is that their interaction

effect of factors. The interaction between average particle size and extraction time is very low with studentized effect of 0.21 and percentage contribution of 0.03%. The interaction between extraction time and pumpkin seed meal to solvent ratio is also low.

Table: 4.8. Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	Remark
Model	2605.96	7	372.28	172.45	< 0.0001	Significant
A	345.04	1	345.04	159.84	< 0.0001	
B	2072.04	1	2072.04	959.84	< 0.0001	
C	176.04	1	176.04	81.55	< 0.0001	
AB	1.04	1	1.04	0.48	0.4949	
AC	9.38	1	9.38	4.34	0.0496	
BC	2.04	1	2.04	0.95	0.3419	
ABC	0.38	1	0.38	0.17	0.6811	
Curvature	869.41	1	869.41	402.74	< 0.0001	Significant
Pure Error	45.33	21	2.16			
Cor Total	3520.70	29				

Table:4.8 show that the model is significant with F-value of 172.45.There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AC are significant model terms. In this case A-particle Size, B-Extraction time, C- meal to solvent ratio and AC- intraction between particle size and pumpkin seed meal to solvent ratio are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant. The P-value of AB and BC (interaction factor) is 0.4949>P-value and 0.3419 >P-value thus; the interactions beteween ; particle size and extraction time and extraction time and solid to solvent ratio are not

significant in the model terms. The "Curvature F-value" of 402.74implies there is significant curvature (as measured difference between the average of the center points and the average of the factorial points) in the design space. There is only a 0.01% chance that a "Curvature F-value" this large could occur due to noise.

Design-expert was applied to analyze results on the extraction process and a first order regression equation, with the interaction terms, of the form, the final model equation in terms of coded factor was presented by equations representing the variation of percentage oil yield of pumpkin seed with independent factors.

4.6.1. Development of regression Model Equation

The model equation that correlates the yield of pumpkinseed oil parameters in terms of coded factors and actual factors was given below in equation (4.1) and (4.2), respectively.

Final Equation in Terms of Coded Factors: expianed in equation 4.1 below

Yield =+76.21-3.79 * A+9.29 * B-2.71 * C - 0.21 * A * B+0.63 * A * C-0.29 * B * C-0.13 * A * B * C (4.1)

Considering ANOVA table 4.4 the model terms A ,B ,C and AC the particle size ,the extraction time and the solid to solvent ratio and interaction beteween particle size and solid to solvent ratio were significant model terms whereas interaction model terms AB and BC are not significant model terms.

The hierarchy principle indicates that if a model contains a high order term, it should contain all lower-order terms that compose it. Hierarchy promotes a type of internal consistency in a model and many statistical model builders rigorously follow the principle. Their for removing non-significant model terms or factors AB and BC from a model are will result in a model that is hierarchal.

Therefore, the final equation in terms of coded factor without non significant interaction effect in order to suport hierarchy is given by a first order regression equation:

Yield =+76.21-3.79 * A+9.29 * B-2.71 * C +0.63 * A * C (4.2)

It is evident from equation 4.2 that the coefficient of [A] and [C] were negative and that of [B] was positive. Therefore increasing the particle size, solid to solvent ratio and decrearesing of extraction time will decrease the percentage oil yield of pumpkin seed.

Final Equation in Terms of Actual Factors

Yield =+71.71429-6.85714 * Particle size+4.9761* Extraction time-130.00000 * Meal:Solvent ratio +40.0000* Particle size * Meal:Solvent ratio (4.3)

Table: 4.9. The regression Coefficient estimate of the process variable and corresponding 95% CI Low and High.

Factor	Coefficient Estimate	DF	Standard Error	95%CI Low	95 %CI High	VIF
Intercept	76.21	1	0.30	75.58	76.83	
A-Particle size	-3.79	1	0.30	4.42	-3.17	1.00
B-Extraction time	9.29	1	0.30	8.67	9.92	1.00
C-Meal:Solvent ratio	-2.71	1	0.30	-3.33	-2.08	1.00
AB	-0.21	1	0.30	-0.83	0.42	1.00
AC	0.63	1	0.30	1.299E-003	1.35	1.00
BC	-0.29	1	0.30	-0.92	0.33	1.00
ABC	-0.13	1	0.30	-0.75	0.5	1.00
Center Point	13.46	1	0.67	12.06	14.85	1.00

4.6.2. Model Adequacy Checking

The model was tested for adequacy by analysis of variance. The regression model was found to be highly significant with the correlation coefficient of determination of R-Squared, adjusted R-Squared and predicted R-Squared of 0.9828, 0.9771 and 0.9734, respectively.

Std. Dev.	1.47	R-Squared	0.9829
Mean	78.90	Adj R-Squared	0.9772
C.V.	1.86	Pred R-Squared	0.9736
PRESS	92.82	Adeq Precision	38.936

PRESS is an acronym for Prediction Error Sum of Squares and it is defined as the sum of squares the differneces between each observations y_i and the corresponding predicted value based on the model fit to the remaining n-1, say mean of y_i , used to evaluate competing regression models. Models that have small values of PRESS are preffered. So this implies that PRESS value of 92.82 is in a prefferable range for this model. C.V. is the coefficent of variation which measures the unexplained or residual variability in the data as a precentage of the mean of response variable.

The "Pred R-Squared" of 0.9736 is in reasonable agreement with the "Adj R-Squared" of 0.9772."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. This study has a ratio of 38.936 indicates an adequate signal. This model can be used to navigate the design space.

The quality of the Model developed could be evaluated from their coefficients of determination. The value of R-Squared for the developed correlation is 0.9829. It implies that 98.28% of total variation in the percentage yield of pumpkin seed oil is attributed to the experimental variables studied. As it is seen from graph 4.8 below, normal probability plot versus the residuals results shows us that the regression model equation provided a very accurate description of experimental data, in which all the data points are very close to the line of perfect fit.

The adequacy of the Model was further checked with analysis of variance (ANOVA) as shown in Table 4.5, based on 95% confidence interval, F-value is a test for comparing model variance with residual (error) variance. If the variances are close to the same, the ratio will be close to unity and it is likely that any factors have a significant effect on the response with the P-value

less than 0.05. It is calculated by model mean square divided by residual mean square. Here the model F-value of 172.45 implies that the model is significant. There is only 0.01% chance that a "Model F Vvalue"this large could occur due to personal error or noise.

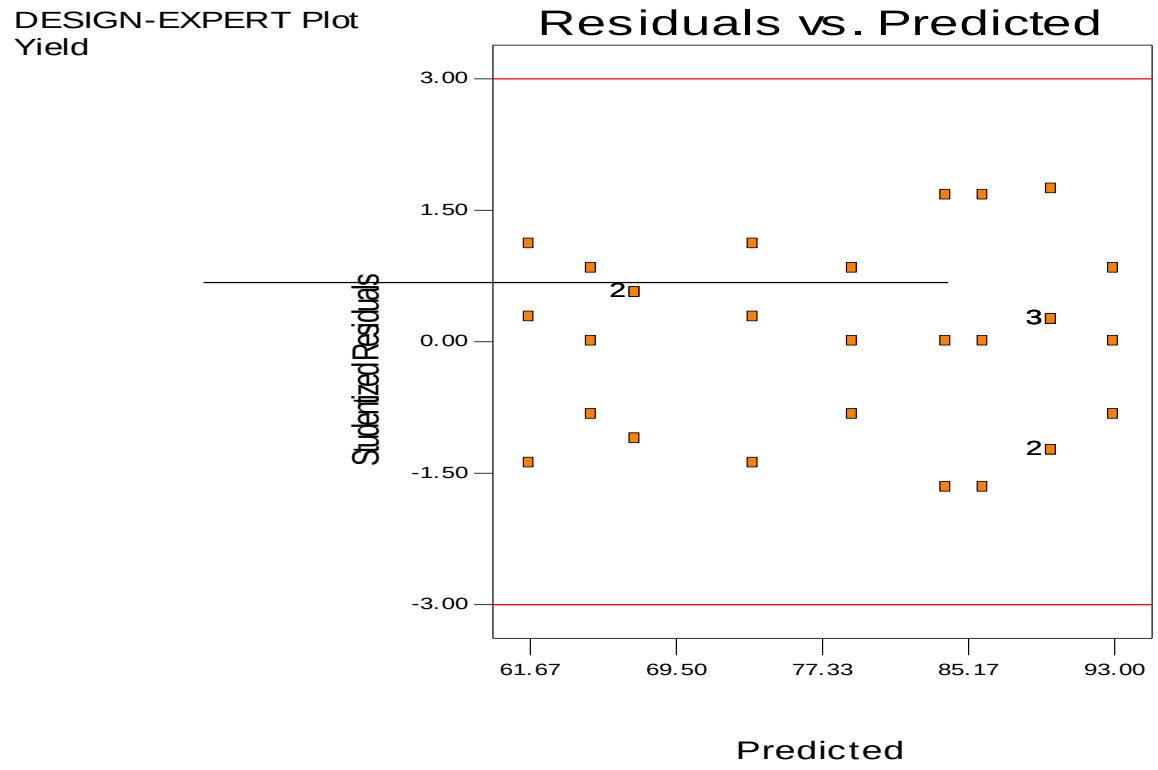


Figure: 4.3. Studentized Residuals versus Predicted Values of yield pumpkin seed oil

From figure 4.3 it was shown that the studentised residuals versus the predicted yield was structureless and scattered so that the conducted experiment was in the acceptable result.

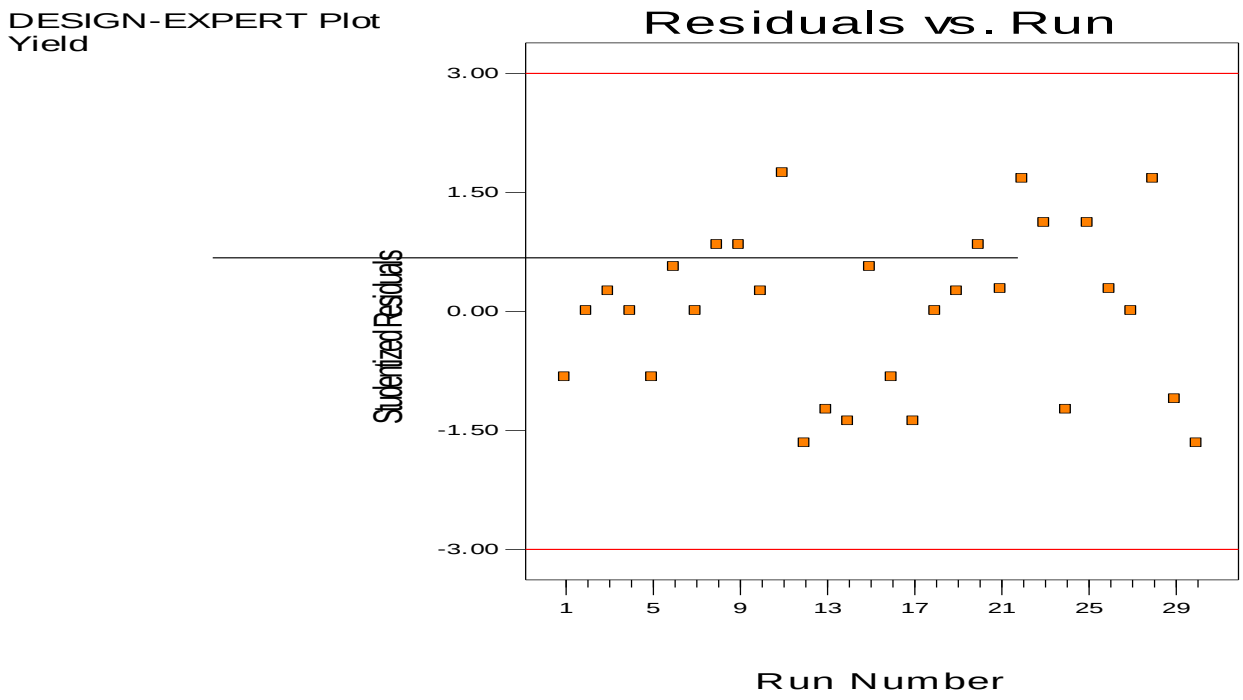


Figure: 4.4. Studentized Residuals versus Run numbers for percentage yield of oil.

As it is seen from figure 4.4 the studentized residuals versus the run numbers were not have a uniform structure which tells us that the experiment was conducted at a randomized design. Figure 4.4 shows us that the normal % probability versus studentized residuals. As it is seen from the graph, the experimental data points in the plot shows that the variance is almost the same and fits with the straight line, approving the linear equation for the yield by ignoring non significant terms interms of model coeffocient esitmate from the model equation. The tendency of the normal probability plot to bend down slightly on the left side implies that the left tail of the error distribution is somewhat thinner than would be anticipated in normal distribution; that is the negative residuals are not quite as large as expected. An error distribution that has considerably thicker or thinner tails than the normal is of more concenrn than a skewed disribution, because the F test is only slightly affect and we may can say that the analysis of variance is robust to the normality assumption.

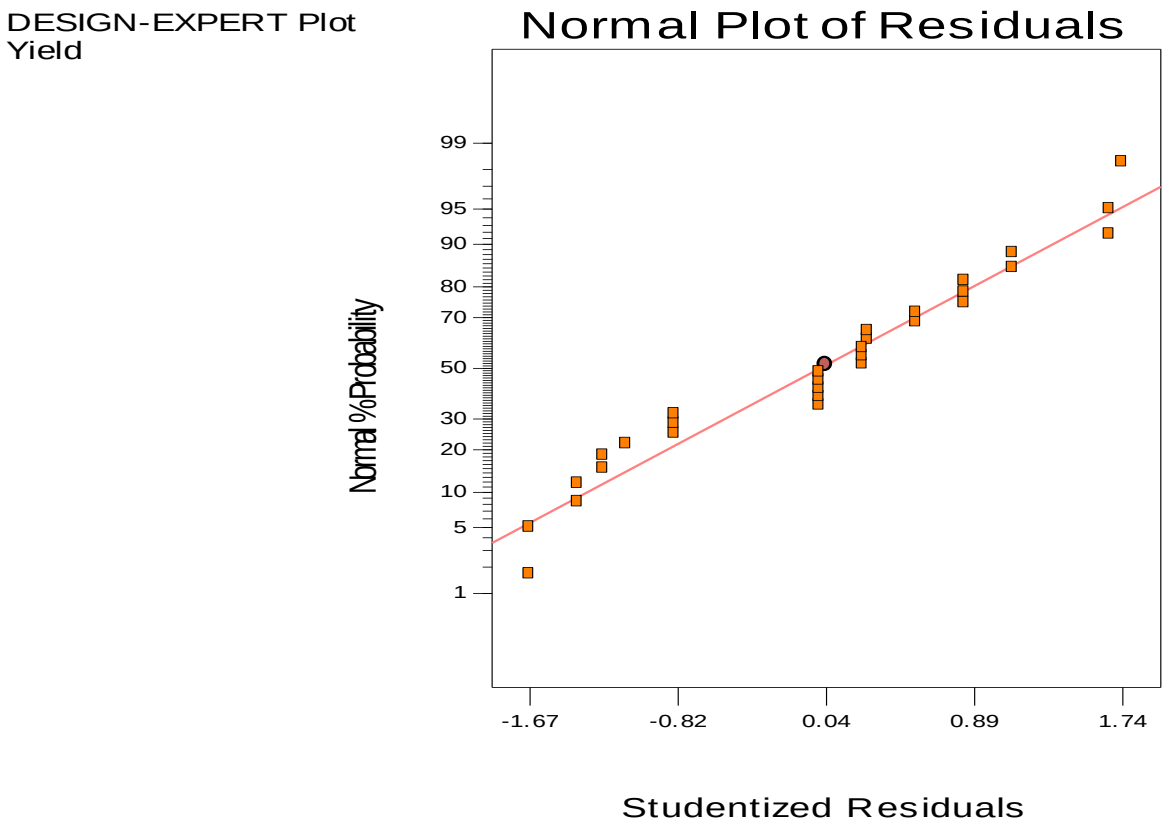


Figure: 4.5. Normal plot of residuals versus studentized residual for percentage oil yield

4.6.3. Effect of process parameter on percentage oil yield

4.6.3.1.Effect of particle size on percentage oil yield

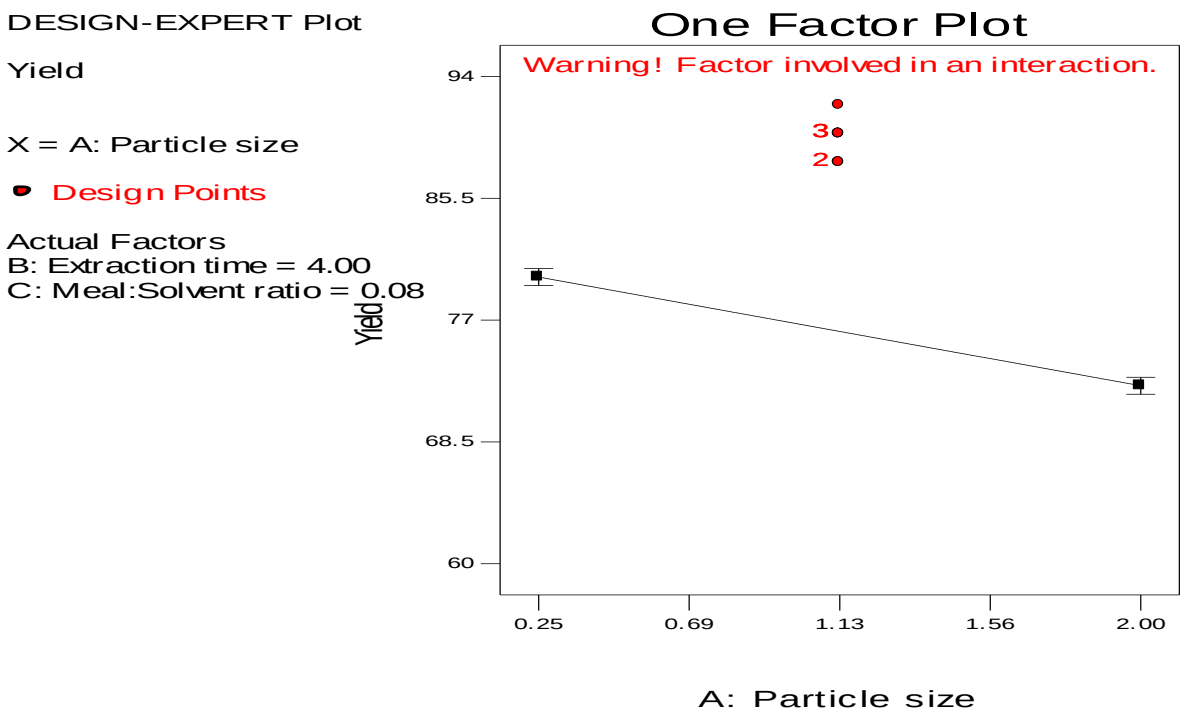


Figure: 4.6. Effect of particle size on percentage oil yield

An increasing the average particle size of pumpkin seed from 0.25 to 1.13 mm decreased the oil yield from 80 to 77% for solvent extraction using ethanol. Similarly as increasing the particle size range from 1.13 to 2 mm decrease the oil yield from 77 to 72.4%. From figure: 4.6. quite clear that there is an increase in the oil yield as the particle size decreased and an increase in the particle size results in a drop in oil yield. Thus, the percentage pumpkin seed essential oil yield was inversely related to the particle size i.e. smaller size gives high yield while larger particle size results a lower yield. The reason is that larger particles have smaller surface area of contact and larger distance to solvent entrance and oil diffusion in comparison to smaller particle using ethanol solvent. Said, (2014), also reported that the oil inside the cells will be more accessible to the solvent, and resulting to increase the extraction yield. On the other hand the larger particle size has a smaller surface area, and the solvent diffuses through the sample would experience a

higher resistance to extract the oil from the inner part of the intact cells resulting in lower extraction yield of bottle gourd seeds.

4.6.3.2.Effect of extraction time on the percentage oil yield

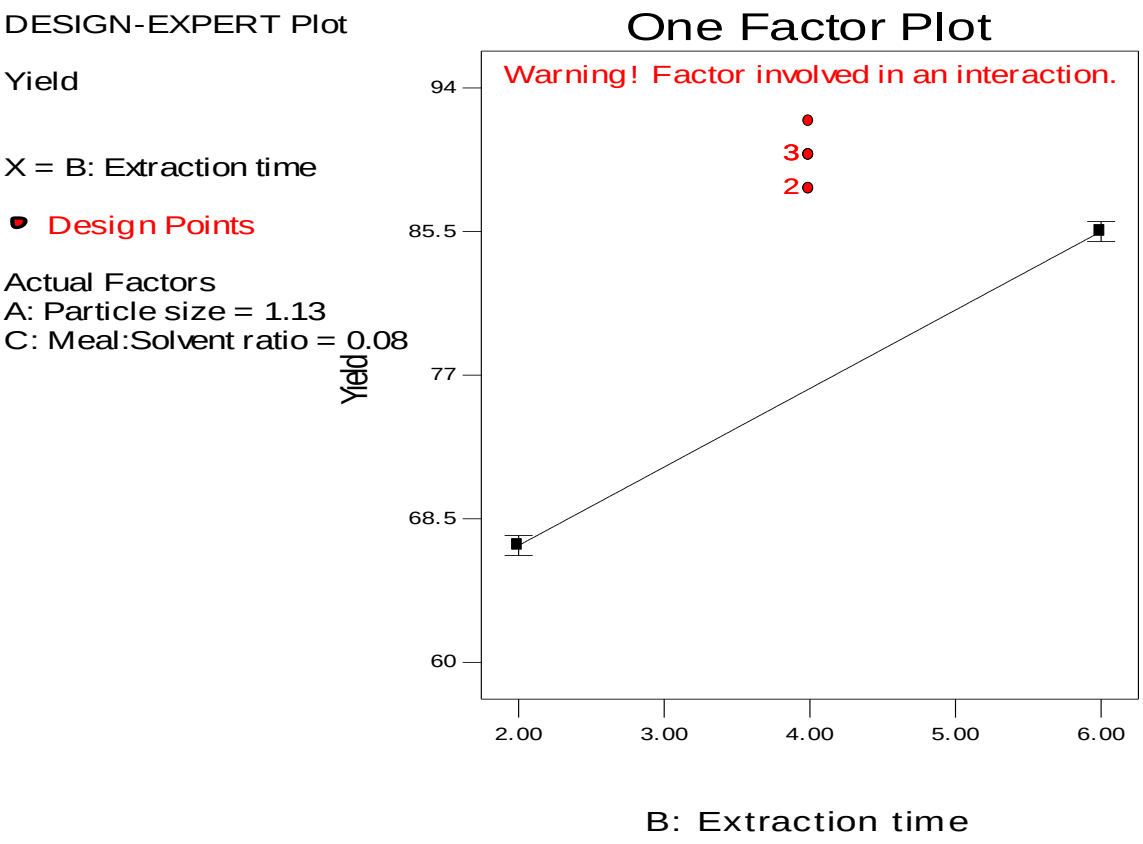


Figure: 4.7 Effect of extraction time on the percentage oil yield

Extraction time was controlled by measuring the time from the observation of first drop of the solvent from the condenser. An increasing the extraction time 2 to 4 hours increase the oil yield from 66.9 to 77% for solvent extraction using ethanol. Similarly as increasing the extraction time from 4 to 6 hours increase the oil yield from 77 to 85.5%. The percentage oil yield was directly related to extraction time i.e. The yield increases as extraction time increases Figure: 4.6 above. This is due to diffusion determines the effect of extraction time. The speed at which equilibrium is reached and the oil extraction rate are influenced by oil diffusion into the solvent,

particle size and internal structure and Longer extraction time favored the system to have more mass transfer (Baldosano, et al., 2015). But more extraction time is note advisable by Fick's second law which is diffusion witch predicts a final equilibrium between the concentrations of solute in the solid matrix and in the bulk solution after a certain time. Therefore, a longer time is not required to extract more extract and utilize more energy.

4.6.3.3.Effect of pumpkin seed meal to solvent (ethanol) ratio on percentage oil yield

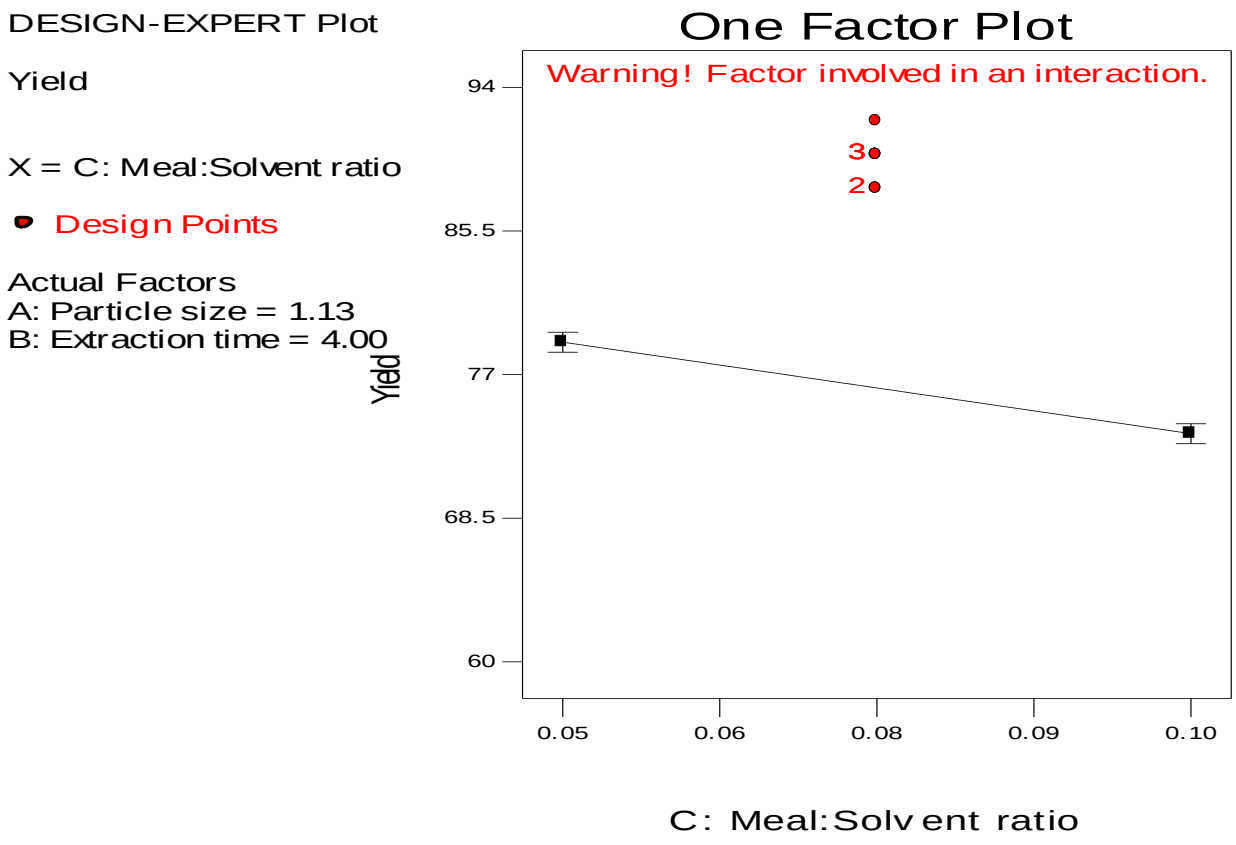


Figure: 4.8. Effect of pumpkin seed meal to solvent (ethanol) ratio on percentage oil yield

Increase pumpkin seed meal to solvent ratio from 0.05 to 0.08 mg/ml decrease the oil yield from 78.9 to 76.5% for solvent extraction. Similarly as increasing the pumpkin seed meal to solvent ratio from 0.08 to 0.01 mg/ml decreasing the oil yield from 76.5 to 73.5%. From Figure: 4.8

shown that the percentage oil yield from pumpkin seed was inversely related to the meal to solvent ratio. Pumpkin seed meal to solvent ratio marks the difference in oil yield up to a certain extent. A high initial extraction rate is attributed to rapid solution of the oil on the solid's surface and a higher conduction mass transference force anticipated by the high solvent concentration. A slower rate can be attributed to a lower motive force resulting from a lower solvent concentration. Extraction with solvents is a mass transfer process in which materials (oils) are moved from one phase to another to separate one or more compounds from a mixture. Pumpkin seed meal to solvent ratio is one of the most important variables in the extraction process,

4.6.4. Interaction effects on percentage oil yield

From design expert software 6.0.8 output, interaction effect between;

- ✓ Particle size and extraction time
- ✓ particle size and pumpkin seed meal to solvent ratio
- ✓ Extraction time and pumpkin seed to solvent ratio

4.6.4.1.Interaction effects of Particle size and extraction time on percentage oil yield

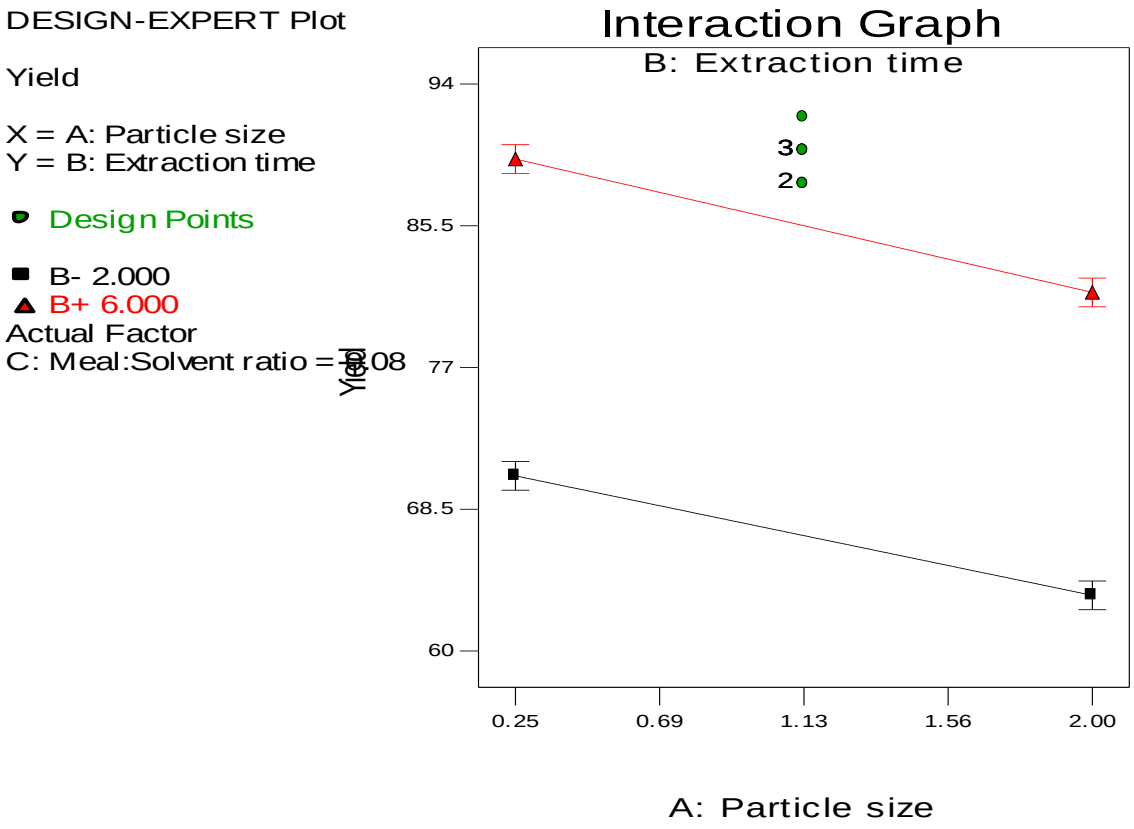


Figure: 4.9. Interaction effects of particle size and extraction time on percentage oil yield

The graphical representation showed that the extraction time and particle size have significant effect on the extraction yield and also there were no interaction between particle size and extraction time as depicted by similar shape of the curves in Figure 4.9. At higher particle size (2 mm), increase in extraction time from 2 hours to 6 hours increased the extraction yield from 63.5 to 80.1%. The same is true for lower particle size (0.25mm); increase in extraction time from 2 hours to 6 hours increased the extraction yield from 70.5 to 89.5%.

This shows that higher extraction time with lower particle sizes give a higher yield and higher particle size with lower extraction time can give lower yield. Similarly as can be noticed from Figure there was no interaction effect is between particle size and extraction time.

Where: A+ and A- are codes for particle sizes ranges 2 mm and 0.25 mm, respectively. B+ and B- are codes for extraction time 6 and 2 hours, with center point 1.13 mm and 4 hours respectively. Design points are points on the graph which helps to develop mathematical model of the predicted response based on these points.

4.6.4.2.Interaction effects of particle size and pumpkin seed meal to solvent ratio on percentage oil yield

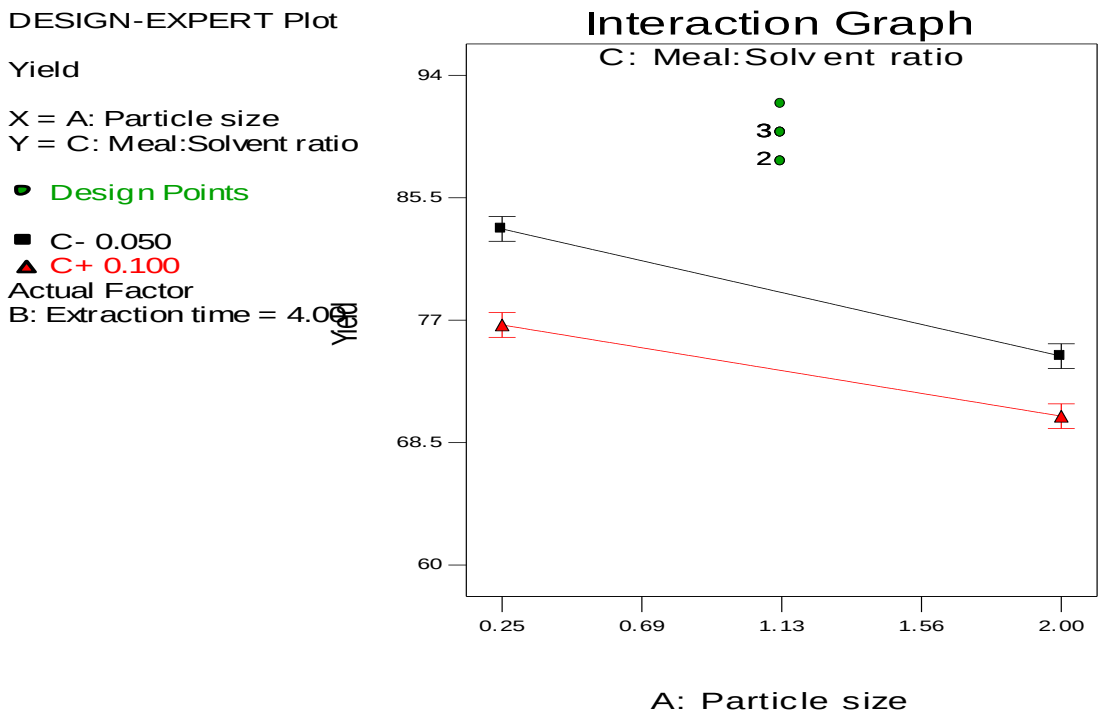


Figure: 4.10. Interaction effects of Particle size and pumpkin seed meal to solvent ratio on percentage oil yield

The graphical representation showed that the particle size and meal to solvent ratio have significant effect on the extraction yield and also there were no interaction between particle size and pumpkin seed meal to solvent ratio as depicted by similar shape of the curves in figure 4.9. At higher particle size (2 mm), decrease in meal to solvent ratio from 0.1mg/ml to 0.05mg/ml increased the extraction yield from 70.5 to 74.35%. The same is true for lower particle size (0.25mm); decrease in meal to solvent ratio from 0.1mg/ml to 0.05mg/ml hours increased the extraction yield from 76.3 to 83%. This shows that lower meal to solvent ratio with

lower particle sizes give a higher yield and higher particle size with higher solid to solvent ratio can give lower yield.

Where: A+ and A- are codes for particle sizes 2 mm and 0.25 mm, respectively. C+ and C- are codes for pumpkin seed meal to solvent ratio 0.1 and 0.05 mg/ml, with center point 1.13 mm and 0.08 mg/ml respectively. Design points are points on the graph which helps to develop mathematical model of the predicted response based on these points.

4.6.4.3. Interaction effects of extraction time and pumpkin seed meal to solvent ratio on percentage oil yield

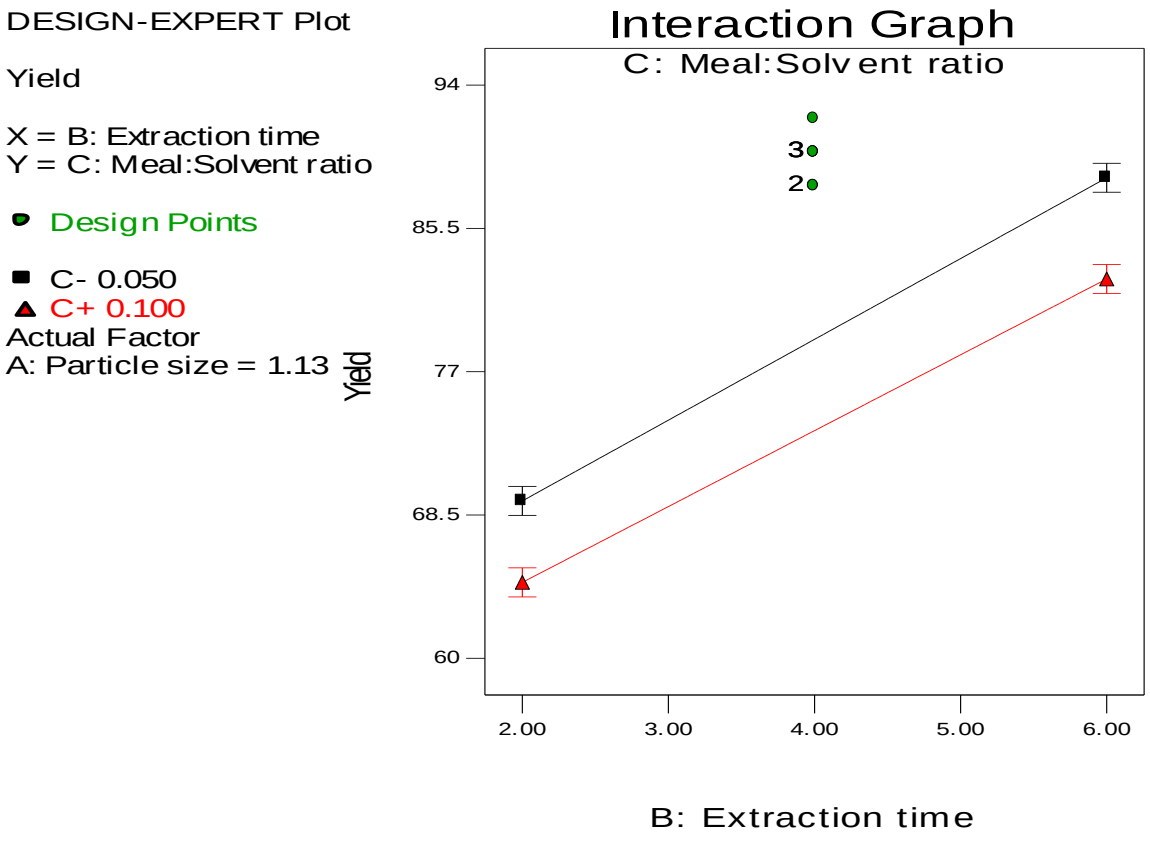


Figure: 4.11. Interaction effects of extraction time and pumpkin seed meal to solvent ratio on percentage oil yield

There were no interaction between extraction time and meal to solvent ratio as depicted by similar shape of the curves in the figure: 4.10. At lower extraction time (2 hours), decrease in

meal to solvent ratio from 0.1mg/ml to 0.05mg/ml increased the extraction yield from 64.5 to 69.66%. The same is true for higher extraction time (6 hours), decrease in meal to solvent ratio from 0.1mg/ml to 0.05mg/ml increased the extraction yield from 82.3 to 88.45%. This shows that higher meal to solvent ratio with lower extraction time give a lower yield and higher extraction time with lower solid to solvent ratio can give higher oil yield.

Where: B+ and B- are codes for particle sizes 6 and 2 hours, respectively. C+ and C- are codes for meal to solvent ratio 0.1 and 0.05 mg/ml, with center point 4 hours and 0.08 mg/ml respectively. Design points are points on the graph which helps to develop mathematical model of the predicted response based on these points.

The following Figure 4.12 shows the relation between the actual predicted values of the experiment by the model equation developed by the design expert software 6.0.8

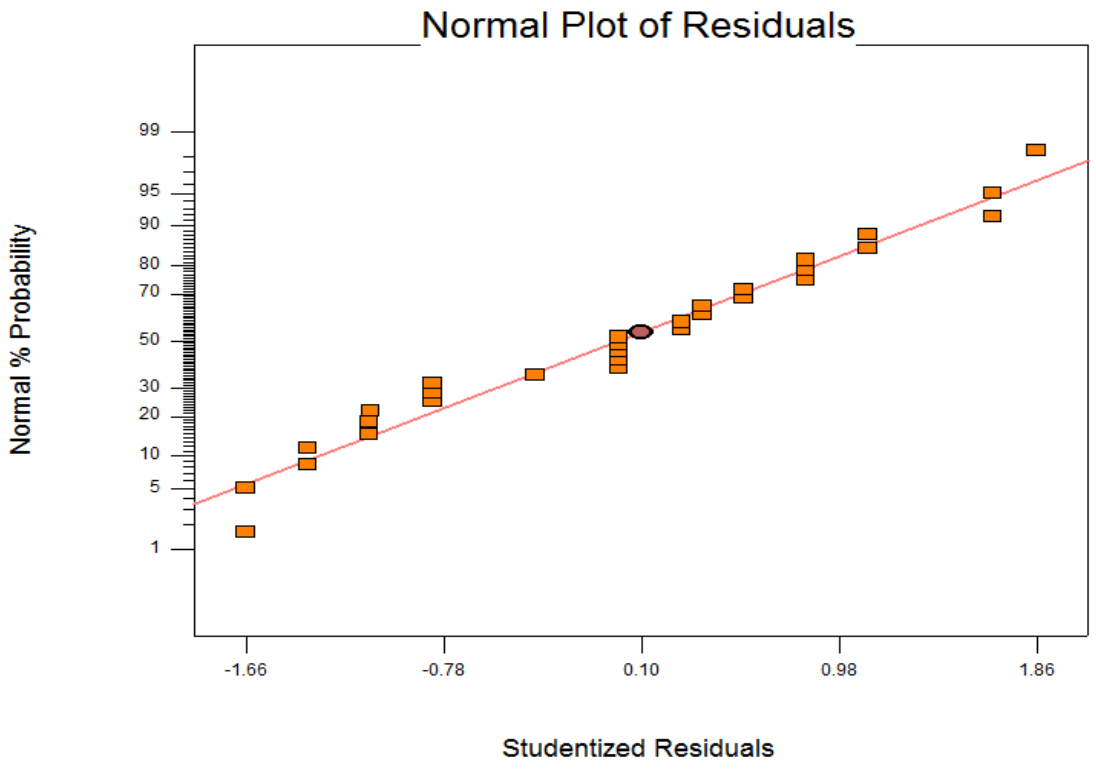


Figure: 4.12. Actual value versus predicated value of percentage oil yield of pumpkin seed

Figures 4.9, 4.10, 4.11 and 4.12 show that there is no interaction among each factor. This shows us an increment in time will increase the quantity of pumpkin seed oil extracted.

Extraction at six hours did give a significant optimum value with the average particle size of 0.25 mm and pumpkin seed meal to solvent ratio 0.05mg/ml on oil yield.

4.7. Optimization

Using optimization functional in design expert software 6.0.8, it was predicted that at the following operating condition; 0.25 mm average particle size, 6 hour extraction time and 0.05g/ml of pumpkin seed meal to solvent ratio, a maximum oil yield 94% was obtained. A minimum oil yield of 74% was predicted at average particle size 2 mm, 2 hour extraction time and 0.1 g/ml pumpkin seed meal to solvent ratio, which was also in agreement with the experimental value because the value of maximum and minimum in oil extraction value subtract from the value of optimized value is less than 0.05.

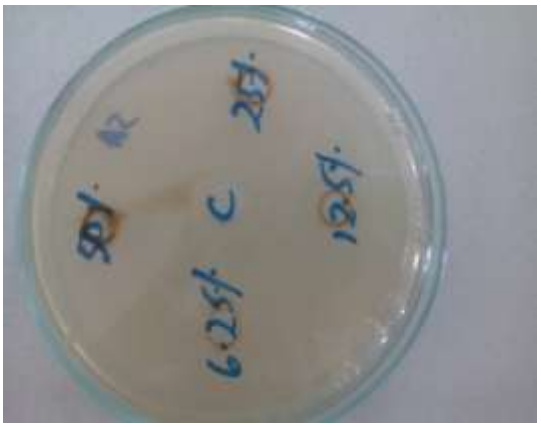
The optimization solutions for maximum yield are shown in Table 4.3. Below by using categorical factor due to the above reason.

Table:4.10. Solutions out put from catagorical optimization for maximum oil yield

Number	Particle size	Time	Meal:solvent ratio	Yield	Desirability	Remark
1	0.25	6.00	0.05	92.997	0.970	Select
2	0.26	6.00	0.05	92.9587	0.969	
3	0.25	5.81	0.05	92.0904	0.944	
4	0.25	6.00	0.06	91.9565	0.940	
5	0.47	6.00	0.05	91.869	0.937	
6	0.25	6.00	0.06	91.0369	0.913	
7	0.25	6.00	0.08	88.5742	0.840	
8	0.25	6.00	0.09	87.0772	0.796	

4.8. Antimicrobial activity of pumpkin seed oil

The assessment of the antimicrobial activity of pumpkin seed oil against pathogenic bacteria (S.aureus and E.coli), shown in Figure: 4.13 A and B that pumpkin seed oil showed strong antibacterial activity against both S.aureus and E.coli.



A B

Figure: 4.13. Sensitivity of (A) Staphylococcus aureus and (B) E.coli on pumpkin seed oil

Table 4:11. Results for the antimicrobial activity essential oil from pumpkin seed oil

Serial dilution	Zone of inhibition mm	
	Staphylococcus aureus	E.coli
1/16	8	7
1/8	9	8.45
1/4	13.3	10
1/2	15.5	13.5
DMSO	0	0

The activity of pumpkin seed oil on S.aureus was shown, the above Table: 4.11. It was average zone of inhibition was 8, 9, 13.3 and 15.5 with receptive serial dilution 1/16, 1/8, 1/4 and 1/2 % v/v. The activity of pumpkin seed oil on E. coli, shown in Table: 4.15. It was average zone of inhibition was 7.5, 8.45, 10.3 and 13.5 with receptive concentration 1/16, 1/8, ¼ and 1/2 % v/v. The activity of Control disc showed 0 zone of inhibition for standard DMSO against pure

bacterial culture of S. aureus and E.coli. Pumpkin seed oil had such compounds which showed strong inhibition in small dilutions against Staphylococcus aureus than E.coli.

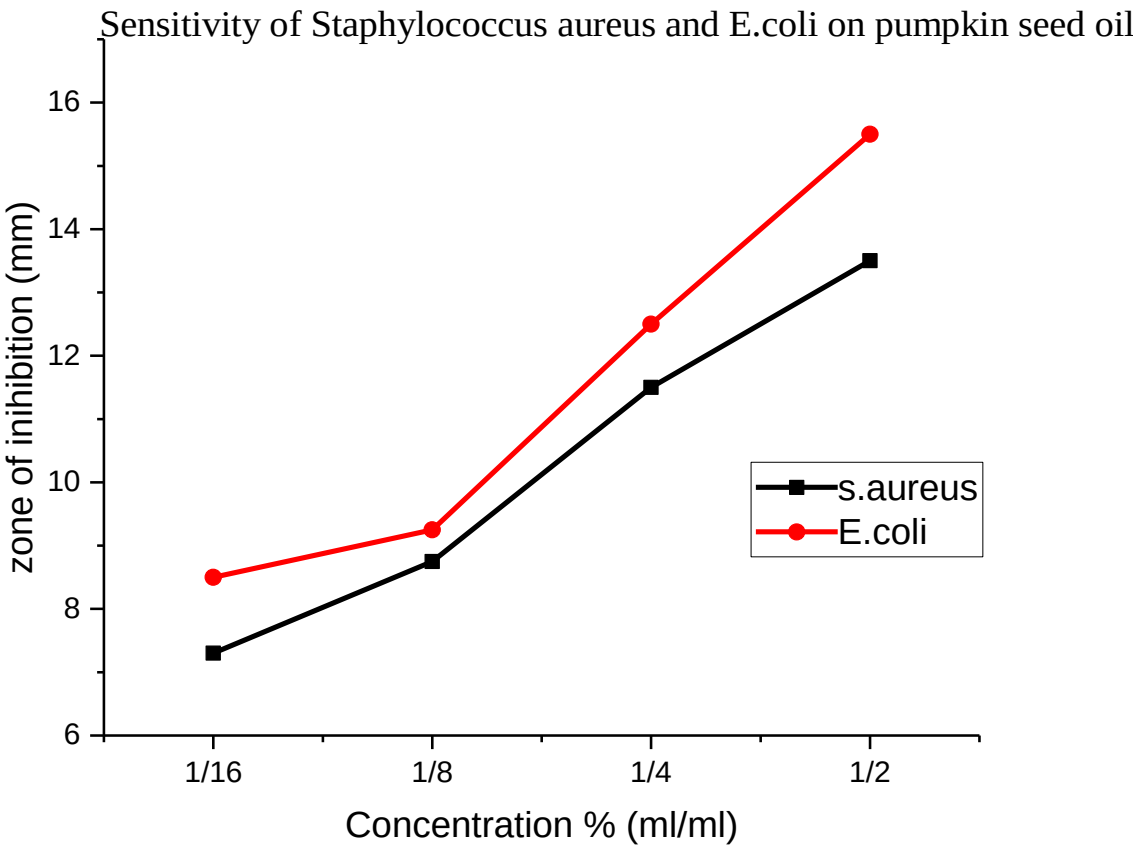


Figure: 4:15. Sensitivity of Staphylococcus aureus and E.coli on pumpkin seed oil

Pumpkin seed oil shown from Table: 4.11 and Figure: 4.15 activities on both phytogenic bacteria. As the concentration of pumpkin seed oil were increased the zone of inhibition were increased on both phytogenic bacteria's. The S. areunes was more sensitive than E.coli which shows more zone of inhibition with respective concentration. This result were closer to the other literatures reported by Del-vechio-vieira, (2009).

4.8.1. Minimum inhibition concentration

The MIC was determined only for oils that presented positive results on bioautographic assays the minimal inhibitory concentration of pumpkin seed oil on S.aureus and E.Coli were done by different serial dilution of 1/16, 1/8, ¼ and 1/2 % (v/v) which is dissolved oil in DMSO on both phytogenic bacteria's. Comparing with literature results, (Sartoratto et al., 2004) strong activity is for MIC values between 1/4-1/2 %(v/v), moderate activity MIC values between 1/8 –1/4 %(v/v) and weak activity above 1/16% (v/v) for both phytogenic bacteria.

The results show a variable effect of the oils on the microorganisms Table: 4.11. Essential oil from Pumpkin seed was active against tested microorganisms, showing the lowest MIC values (1/16 and 1/16 % (v/v) against S.aruens and E.coli respectively.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

This work was intended to study the influence of different factors (Particle sizes, extraction time and pumpkin seed meal to solvent ratio) on the quality and quantity of essential oil extracted from pumpkin seeds using ethanol as solvent and evaluation of pumpkin seed essential oil for antimicrobial activity. Variability of these operating conditions is the pre-dominant factors for the quality and quantity of pumpkin seed essential oil.

There are different methods of essential oil extraction from pumpkin seed. In this thesis, Soxhlet extraction was used. From the experimentation it was found that maximum oil yield of 97% was obtained at average particle size of 0.25 mm, extraction time of 6 hour and pumpkin seed meal to solvent ratio of 0.05mg/ml. A minimum oil yield of 74 % was obtained at average particle size of 2mm, extraction time of 2 hour and pumpkin seed meal to solvent ratio 0.1mg/ml, the observed quantitative difference in the quantity of the oil was due to particle size, extraction time and pumpkin seed meal to solvent ratio. Thus, determination of appropriate size of the particle, optimal extraction time and pumpkin seed meal to solvent ratio for the recommended particle size needs to have a consideration to get the maximum amount of the required product.

From design expert software the analysis of ANOVA P value < 0.0001 for particle size extraction time and pumpkin seed meal to solvent ratio indicate that operating parameters have significant effect on oil yield.

Physiochemical property of extracted oil were specific gravity, pH, kinematic viscosity, density moisture and volatile contents, refractive index, free fatty acid, iodine, acid value and saponification value were: 0.91178, 5.16 $\pm$ 0.1, 35, 911.78, 0.07, 1.468, 0.62$\pm$0.61, 97.5 I_2g/100g oil, 1.23$\pm$ 0.23mg KOH/g oil and 189.8$\pm$ 0.31mg KOH/g oil respectively which are in range of literature. The result obtained was increased as compared it from the literature using ethanol as a solvent. The quality of the oil could be affected due to several reasons like purities with the seed, genotype of the seed, operating conditions, maturity stage, drying condition, and type of soil and extraction equipment. From the investigation, particle size and extraction time was the dominant factor for the change in quality of the oil.

Analysis using Gas Chromatography-Mass Spectrometer was found to be the best method to identify fatty acid composition of particular oil along with major components, and determination of functional group by FTIR. Characterization of essential oil enumerated that polyunsaturated fatty acids were the dominating fraction i.e. 57.5% as compared to saturate and monounsaturated fatty acids i.e. 22.4% and 20.2. Thus, investigation of optimal operating condition has to be taken in to consideration.

The results were compared with commercial available pumpkin seed essential oil Specification and standard except the Moisture and volatile matter of oil which is slightly below the level, other properties in desired

The valuable antimicrobial activities of pumpkin seed oil showed that it is effective against gram positive bacteria S. aureus and gram negative bacteria E.coli. Pumpkin seed essential oil showed a good potential new source of high-value oils.

5.2. Recommendations

Recommendation for further work

- Comparison of different extraction technology such as steam distillation, supercritical fluid extraction and cold press with solvent extraction is suggested.
- Detailed feasibility study of pumpkin seed essential oil from pumpkin seed in Ethiopia is recommended
- Effect of temperature and solvent type and other factors on the percentage oil yield is suggested.
- Pumpkin seed essential oil for cosmetics, shampoo, cream and soap application is suggested
- Anti-microbial activity on other micro-organism such as other bacteria, fungus and virus.
- Further researcher could use as reference for further work on pumpkin seed oil.

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APPENDICES

Appendix: A. Formulas and Equations used for characterization of the oil

1. Moisture content (%) of the Pumpkinseed=$\frac{W1-W2}{W1} \times 100$ A.1

   Where W_1= original weight of the sample before drying

   W_2= weight of the sample after drying

2. Moisture and volatile matter of oil = $\frac{W1}{W0} \times 100\%$ A.2

Where W_1= loss in gram of the material on drying

W_0= weight in gram of oil taken for the test

3. Specific gravity at 30^0C = A.3

Where: A= weight in gm. of density of bottle with oil at 30^0c

B= weight in gm. of density of bottle at 30^0c

C= weight in gm. of density of bottle with water at 30^0c

4. Kinematic viscosity of the oil (V) = $\frac{\mu}{\rho}$ A.4

Where: μ = dynamic viscosity

ρ = density of oil

5. Acid value

Acid value (AV) = $56.11 \times \frac{V \times C}{m}$ A.5

Where: V =Volume of potassium hydroxide (ml)

C=Concentration of potassium hydroxide

56.11 =Molecular weight of potassium hydroxide

m= sample weight

6. Percent free fatty acid (as oleic acid) = A.6

Where: AV= acid value*0.508

7. The saponification value

SV=$56.1 \times \frac{N(VO-VI\ )}{m}$ A.7

Where: V_O = volume of HCl solution used for the blank test

V_I = volume of HCl solution for the determination

N = actual molarity of HCl used

m = mass of sample

8. Percent free fatty acid (as oleic acid) = $\frac{AV}{1.99}$ A.8

Where: AV= acid value

Apindex : B. ANOVA results from design- expert 6.0.8

Table B1: Diagnostics Case Statistics

Standard Order	Actual Value	Predicted Value	Residual	Leverage	Student Residual	Cook's Distance	Outlier t	Run Order
1	37.00	36.83	0.17	0.333	0.277	0.004	0.271	26

2	37.50	36.83	0.67	0.333	1.109	0.068	1.116	23
3	36.00	36.83	-0.83	0.333	-1.387	0.107	-1.420	14
4	32.00	32.50	-0.50	0.333	-0.832	0.038	-0.826	1
5	33.00	32.50	0.50	0.333	0.832	0.038	0.826	9
6	32.50	32.50	0.000	0.333	0.000	0.000	0.000	18
7	47.00	46.50	0.50	0.333	0.832	0.038	0.826	20
8	46.00	46.50	-0.50	0.333	-0.832	0.038	-0.826	5
9	46.50	46.50	0.000	0.333	0.000	0.000	0.000	7
10	41.00	42.00	-1.00	0.333	-1.664	0.154	-1.743	12
11	42.00	42.00	0.000	0.333	0.000	0.000	0.000	4
12	43.00	42.00	1.00	0.333	1.664	0.154	1.743	28
13	34.00	33.67	0.33	0.333	0.555	0.017	0.545	15
14	33.00	33.67	-0.67	0.333	-1.109	0.068	-1.116	29
15	34.00	33.67	0.33	0.333	0.555	0.017	0.545	6
16	30.00	30.83	-0.83	0.333	-1.387	0.107	-1.420	17
17	31.00	30.83	0.17	0.333	0.277	0.004	0.271	21
18	31.50	30.83	0.67	0.333	1.109	0.068	1.116	25
19	44.00	43.00	1.00	0.333	1.664	0.154	1.743	22
20	43.00	43.00	0.000	0.333	0.000	0.000	0.000	27
21	42.00	43.00	-1.00	0.333	-1.664	0.154	-1.743	30
22	39.00	39.50	-0.50	0.333	-0.832	0.038	-0.826	16
23	39.50	39.50	0.000	0.333	0.000	0.000	0.000	2
24	40.00	39.50	0.50	0.333	0.832	0.038	0.826	8
25	44.00	44.75	-0.75	0.167	-1.116	0.028	-1.123	24
26	45.00	44.75	0.25	0.167	0.372	0.003	0.364	3
27	44.50	44.75	-0.25	0.167	-0.372	0.003	-0.364	10
28	46.00	44.75	1.25	0.167	1.861	0.077	1.987	11
29	44.00	44.75	-0.75	0.167	-1.116	0.028	-1.123	13
30	45.00	44.75	0.25	0.167	0.372	0.003	0.364	19

Table: B2. Constraints for optimization for maximum percentage yield of oil from pumpkin seed oil

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Particle size	is in range	0.25	2	1	1	3
Extraction time	is in range	2	6	1	1	3
Meal:Solvent ratio	is in range	0.05	0.1	1	1	3
Yield	maximize	30	47	1	1	3

Appendix: C. Functional groups which displayed on IR

Table: C1. Functional groups which displayed on IR

Functional Group	Vibration	Characteristic Absorptions (cm-1)	Intensity

Alcohol			
O-H	(stretch, H-bonded)	3200-3600	strong, broad
O-H	(stretch, free)	3500-3700	strong, sharp
C-O	(stretch)	1050-1150	strong
Alkane			
C-H	stretch	2850-3000	strong
-C-H	bending	1350-1480	variable
Alkene			
=C-H	stretch	3010-3100	medium
=C-H	bending	675-1000	strong
C=C	stretch	1620-1680	variable
Alkyl Halide			
C-F	stretch	1000-1400	strong
C-Cl	stretch	600-800	strong
C-Br	stretch	500-600	strong
C-I	stretch	500	strong
Alkyne			
C-H	stretch	3300	strong, sharp
	stretch	2100-2260	variable, not present in symmetrical alkynes
Amine			

N-H	stretch	3300-3500	medium (primary amines have two bands; secondary have one band, often very weak)
C-N	stretch	1080-1360	medium-weak
N-H	bending	1600	medium
Aromatic			
C-H	stretch	3000-3100	medium
C=C	stretch	1400-1600	medium-weak, multiple bands
Analysis of C-H out-of-plane bending can often distinguish substitution patterns			
Carbonyl			
C=O	stretch	1670-1820	strong
(conjugation moves absorptions to lower wave numbers)			
Ether			
C-O	stretch	1000-1300 (1070-1150)	strong
Nitrile			
CN	stretch	2210-2260	medium
Nitro			
N-O	stretch	strong, two bands	

Appendix: D. Fatty acid composition of pumpkin seed oil

Table: D1: Library search report of GC-MS for pumpkin seed oil

LIDI

LEATHER INDUSTRY DEVELOPMENT INSTITUTE
TESTING & RESAERCH LABORATORY
DIRECTORATE

TEST REPORT

Test date(s) : 16/04/18
Report No : C-13193 to C-13195/18
Lab. Desg .Code No : C-13193 to C-13195
Test order No: SCBE-396/2010
Type of Sample : OIL
Sample Identification : --
Sampling : By Customer
Conditioning : Room temperature.
Sample extraction : By Customer
Sampling date & place : ---
Sampling location : ---
Sample receiving date : 09/03/18
Sampled by : Customer
Report date : 18/04/18

Environmental test condition: Temp.(°C) 22.1 R/H (%) 54.1

ORIGINAL

Name of Customer: ADDIS ABABA INSTITUTE OF TECHNOLOGY,
ADDIS ABABA UNIVERSITY, SCHOOL OF CHEMICAL AND BIO-ENGINEERING

Address of customer: Tel.(251)1232417
Fax 011-1239480
P.O.Box 385
Email:info@aau.edu.et

Used equipment/ instruments:- Gas Chromatograph (GC-MS)

S/No	Type of test	Lab code	Customer code	Unit	Test Result	Uncertainty	Test method	Standard Requ.	Rem
1.	Oil	C-13193	Wondifraw	%	Attached	-	By customer	-	
2.		C-13194	Pumpkinseed	%	Attached	-		-	
3.		C-13195	Tilahun	%	Attached	-		-	

Note: **1. Batch no 13194&13195 Sample was diluted 50 times prior to analysis on GC-MS rather batch no 13193 was not further diluted**
The test results relates only to the item tested
This test report is for technical information of the client only. Not for the advisement, promo
ublicity litigation or legal purpose.

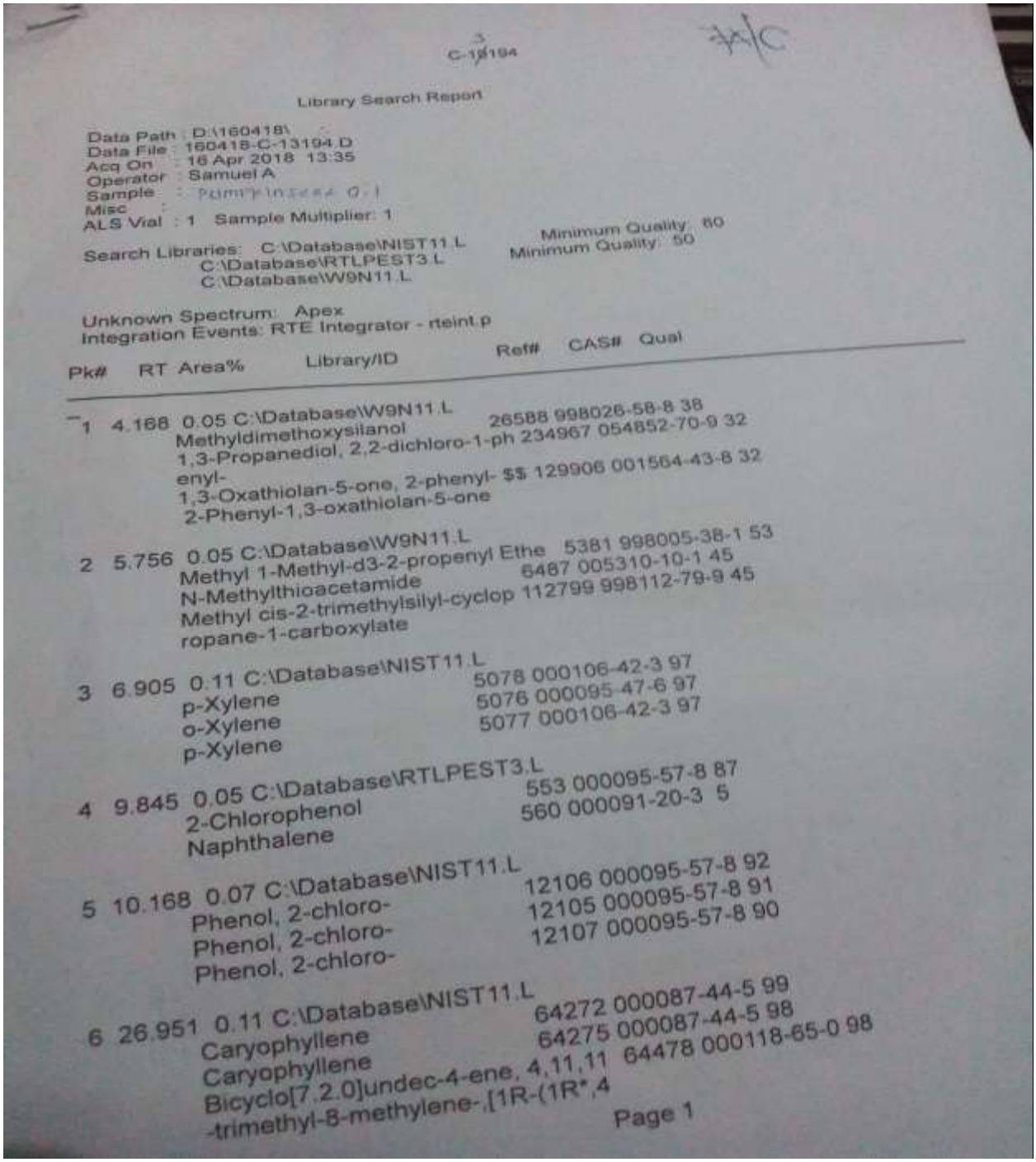
Tested By: Samuel A. Signature
Chemical analysis Expert IV

Checked By: Meron M. Signature
Lead Chemical Analysis Expert (Team Leader)

Authorized By: Berhanu Negus Baraki
Director,Research & Testi
Laboratory Directorate

+251-11-439 1700, +251-11-439 4846 24 692(1000) Fax: +251-11-439 2259 E-mail:berhanunegus@gmail.com
Mobile +251-911 252713 elidilab@gmail.com

AKAKI-KALITY KEFLE KETEMA, ADDIS ABABA



C-13194

Library Search Report

Data Path : D:\160418\
Data File : 160418-C-13194.D
Acq On : 16 Apr 2018 13:35
Operator : Samuel A
Sample : Pumpkin seed 0.1
Misc :
ALS Vial : 1 Sample Multiplier: 1

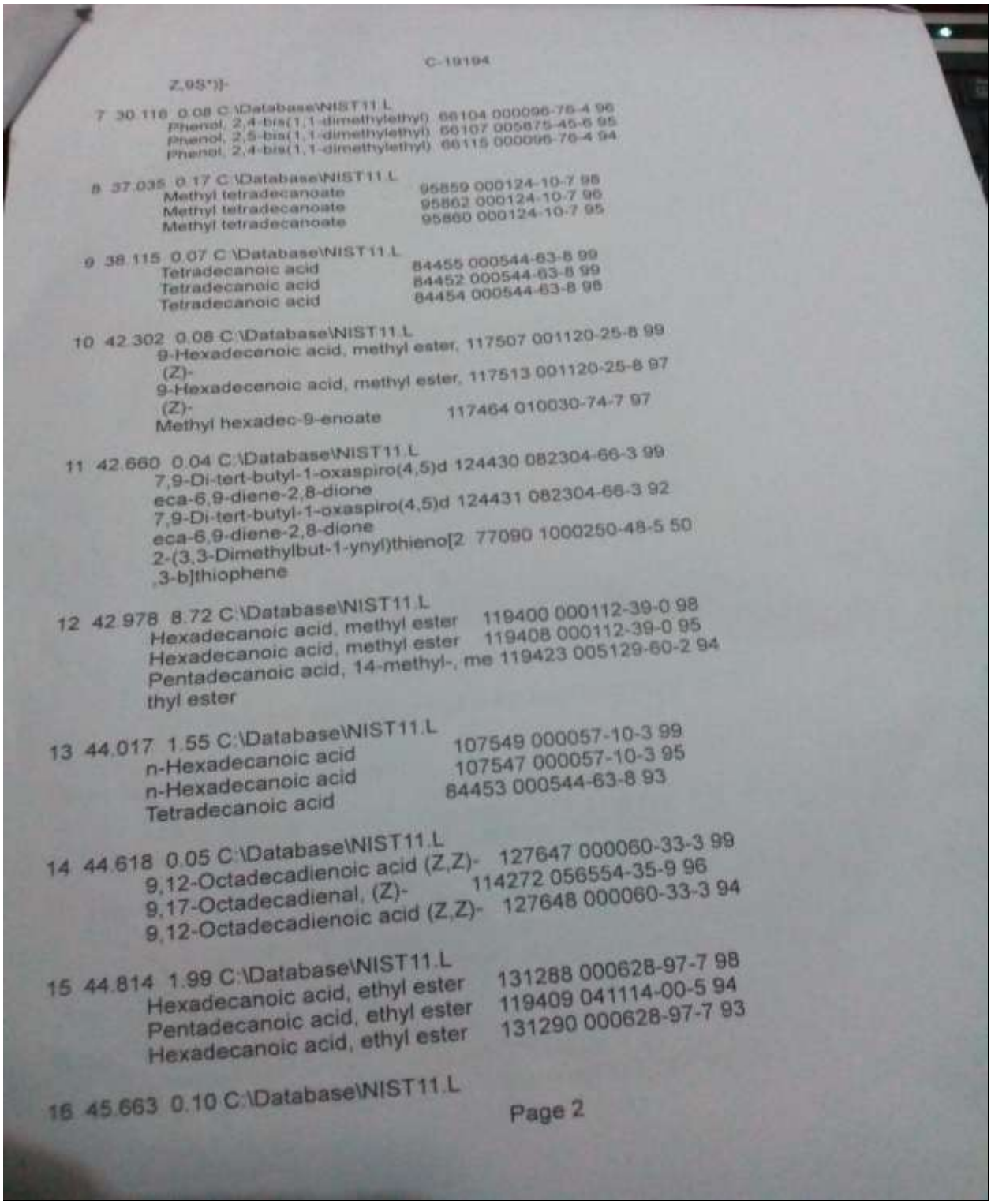
Search Libraries: C:\Database\NIST11.L
C:\Database\RTLPEST3.L
C:\Database\W9N11.L

Minimum Quality: 60
Minimum Quality: 50

Unknown Spectrum: Apex
Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	4.168	0.05	C:\Database\W9N11.L			
			Methyldimethoxysilanol	26588	998026-58-8	38
			1,3-Propanediol, 2,2-dichloro-1-ph	234967	054852-70-9	32
			enyl-			
			1,3-Oxathiolan-5-one, 2-phenyl- $$	129906	001564-43-8	32
			2-Phenyl-1,3-oxathiolan-5-one			
2	5.756	0.05	C:\Database\W9N11.L			
			Methyl 1-Methyl-d3-2-propenyl Ethe	5381	998005-38-1	53
			N-Methylthioacetamide	6487	005310-10-1	45
			Methyl cis-2-trimethylsilyl-cyclop	112799	998112-79-9	45
			ropane-1-carboxylate			
3	6.905	0.11	C:\Database\NIST11.L			
			p-Xylene	5078	000106-42-3	97
			o-Xylene	5076	000095-47-6	97
			p-Xylene	5077	000106-42-3	97
4	9.845	0.05	C:\Database\RTLPEST3.L			
			2-Chlorophenol	553	000095-57-8	87
			Naphthalene	560	000091-20-3	5
5	10.168	0.07	C:\Database\NIST11.L			
			Phenol, 2-chloro-	12106	000095-57-8	92
			Phenol, 2-chloro-	12105	000095-57-8	91
			Phenol, 2-chloro-	12107	000095-57-8	90
6	26.951	0.11	C:\Database\NIST11.L			
			Caryophyllene	64272	000087-44-5	99
			Caryophyllene	64275	000087-44-5	98
			Bicyclo[7.2.0]undec-4-ene, 4,11,11	64478	000118-65-0	98
			-trimethyl-8-methylene-,[1R-(1R*,4			

Page 1



C-19194

Z,9S*)]-

7 30.116 0.08 C:\Database\NIST11.L
Phenol, 2,4-bis(1,1-dimethylethyl) 66104 000096-76-4 96
Phenol, 2,5-bis(1,1-dimethylethyl) 66107 005875-45-6 95
Phenol, 2,4-bis(1,1-dimethylethyl) 66115 000096-76-4 94

8 37.035 0.17 C:\Database\NIST11.L
Methyl tetradecanoate 95859 000124-10-7 98
Methyl tetradecanoate 95862 000124-10-7 96
Methyl tetradecanoate 95860 000124-10-7 95

9 38.115 0.07 C:\Database\NIST11.L
Tetradecanoic acid 84455 000544-63-8 99
Tetradecanoic acid 84452 000544-63-8 99
Tetradecanoic acid 84454 000544-63-8 98

10 42.302 0.08 C:\Database\NIST11.L
9-Hexadecenoic acid, methyl ester, 117507 001120-25-8 99
(Z)-
9-Hexadecenoic acid, methyl ester, 117513 001120-25-8 97
(Z)-
Methyl hexadec-9-enoate 117464 010030-74-7 97

11 42.660 0.04 C:\Database\NIST11.L
7,9-Di-tert-butyl-1-oxaspiro(4,5)d 124430 082304-66-3 99
eca-6,9-diene-2,8-dione
7,9-Di-tert-butyl-1-oxaspiro(4,5)d 124431 082304-66-3 92
eca-6,9-diene-2,8-dione
2-(3,3-Dimethylbut-1-ynyl)thieno[2 77090 1000250-48-5 50
,3-b]thiophene

12 42.978 8.72 C:\Database\NIST11.L
Hexadecanoic acid, methyl ester 119400 000112-39-0 98
Hexadecanoic acid, methyl ester 119408 000112-39-0 95
Pentadecanoic acid, 14-methyl-, me 119423 005129-60-2 94
thyl ester

13 44.017 1.55 C:\Database\NIST11.L
n-Hexadecanoic acid 107549 000057-10-3 99
n-Hexadecanoic acid 107547 000057-10-3 95
Tetradecanoic acid 84453 000544-63-8 93

14 44.618 0.05 C:\Database\NIST11.L
9,12-Octadecadienoic acid (Z,Z)- 127647 000060-33-3 99
9,17-Octadecadienal, (Z)- 114272 056554-35-9 96
9,12-Octadecadienoic acid (Z,Z)- 127648 000060-33-3 94

15 44.814 1.99 C:\Database\NIST11.L
Hexadecanoic acid, ethyl ester 131288 000628-97-7 98
Pentadecanoic acid, ethyl ester 119409 041114-00-5 94
Hexadecanoic acid, ethyl ester 131290 000628-97-7 93

16 45.663 0.10 C:\Database\NIST11.L

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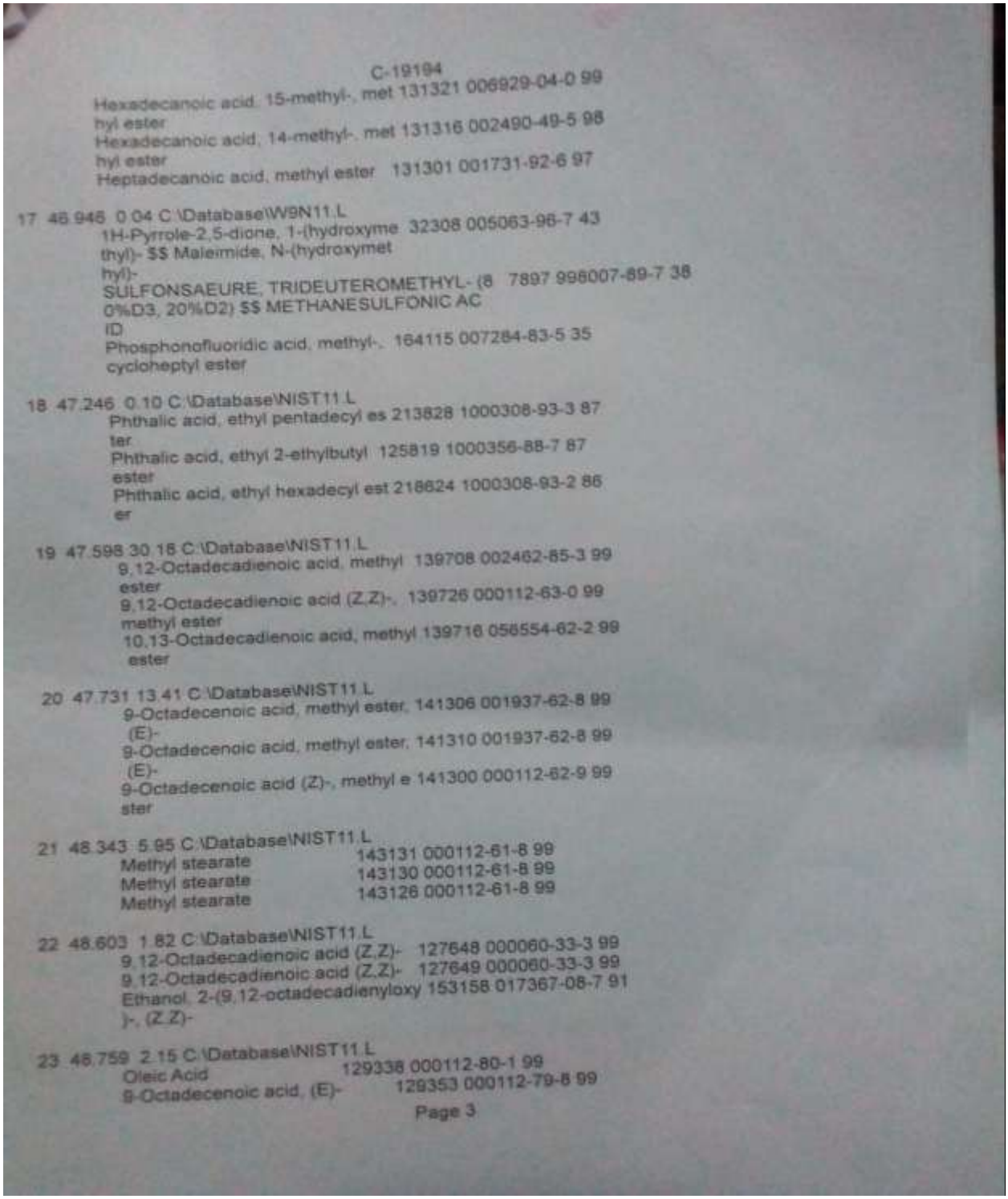

C-19194
Hexadecanoic acid, 15-methyl-, met 131321 006929-04-0 99
hyl ester
Hexadecanoic acid, 14-methyl-, met 131316 002490-49-5 98
hyl ester
Heptadecanoic acid, methyl ester 131301 001731-92-6 97

17 46.946 0.04 C:\Database\W9N11.L
1H-Pyrrole-2,5-dione, 1-(hydroxyme 32308 005063-96-7 43
thyl)- $$ Maleimide, N-(hydroxymet
hyl)-
SULFONSAEURE, TRIDEUTEROMETHYL- (8 7897 998007-89-7 38
0%D3, 20%D2) $$ METHANESULFONIC AC
ID
Phosphonofluoridic acid, methyl-, 164115 007284-83-5 35
cycloheptyl ester

18 47.246 0.10 C:\Database\NIST11.L
Phthalic acid, ethyl pentadecyl es 213828 1000308-93-3 87
ter
Phthalic acid, ethyl 2-ethylbutyl 125819 1000356-88-7 87
ester
Phthalic acid, ethyl hexadecyl est 218824 1000308-93-2 86
er

19 47.598 30.16 C:\Database\NIST11.L
9,12-Octadecadienoic acid, methyl 139708 002462-85-3 99
ester
9,12-Octadecadienoic acid (Z,Z)-, 139726 000112-63-0 99
methyl ester
10,13-Octadecadienoic acid, methyl 139716 056554-62-2 99
ester

20 47.731 13.41 C:\Database\NIST11.L
9-Octadecenoic acid, methyl ester, 141306 001937-62-8 99
(E)-
9-Octadecenoic acid, methyl ester, 141310 001937-62-8 99
(E)-
9-Octadecenoic acid (Z)-, methyl e 141300 000112-62-9 99
ster

21 48.343 5.95 C:\Database\NIST11.L
Methyl stearate 143131 000112-61-8 99
Methyl stearate 143130 000112-61-8 99
Methyl stearate 143126 000112-61-8 99

22 48.603 1.82 C:\Database\NIST11.L
9,12-Octadecadienoic acid (Z,Z)- 127648 000060-33-3 99
9,12-Octadecadienoic acid (Z,Z)- 127649 000060-33-3 99
Ethanol, 2-(9,12-octadecadienyloxy 153158 017367-08-7 91
)-, (Z,Z)-

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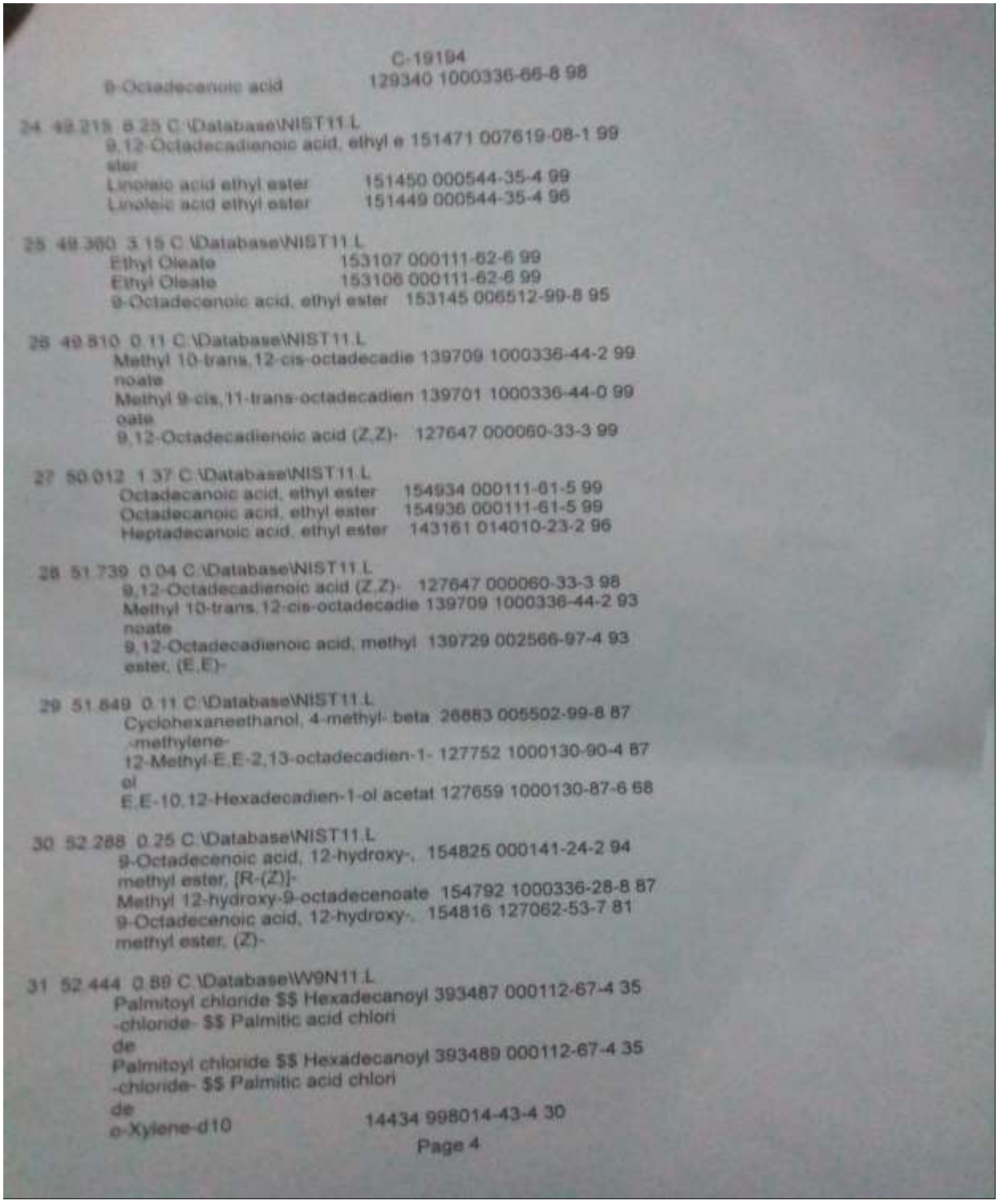
Oleic Acid 129338 000112-80-1 99
9-Octadecenoic acid, (E)- 129353 000112-79-8 99

Page 3



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9-Octadecenoic acid 129340 1000336-66-8 98

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9,12-Octadecadienoic acid, ethyl e 151471 007619-08-1 99
ster
Linoleic acid ethyl ester 151450 000544-35-4 99
Linoleic acid ethyl ester 151449 000544-35-4 96

25 48.360 3.15 C:\Database\NIST11.L
Ethyl Oleate 153107 000111-62-6 99
Ethyl Oleate 153106 000111-62-6 99
9-Octadecenoic acid, ethyl ester 153145 006512-99-8 95

26 49.810 0.11 C:\Database\NIST11.L
Methyl 10-trans,12-cis-octadecadie 139709 1000336-44-2 99
noate
Methyl 9-cis,11-trans-octadecadien 139701 1000336-44-0 99
oate
9,12-Octadecadienoic acid (Z,Z)- 127647 000060-33-3 99

27 50.012 1.37 C:\Database\NIST11.L
Octadecanoic acid, ethyl ester 154934 000111-61-5 99
Octadecanoic acid, ethyl ester 154936 000111-61-5 99
Heptadecanoic acid, ethyl ester 143161 014010-23-2 96

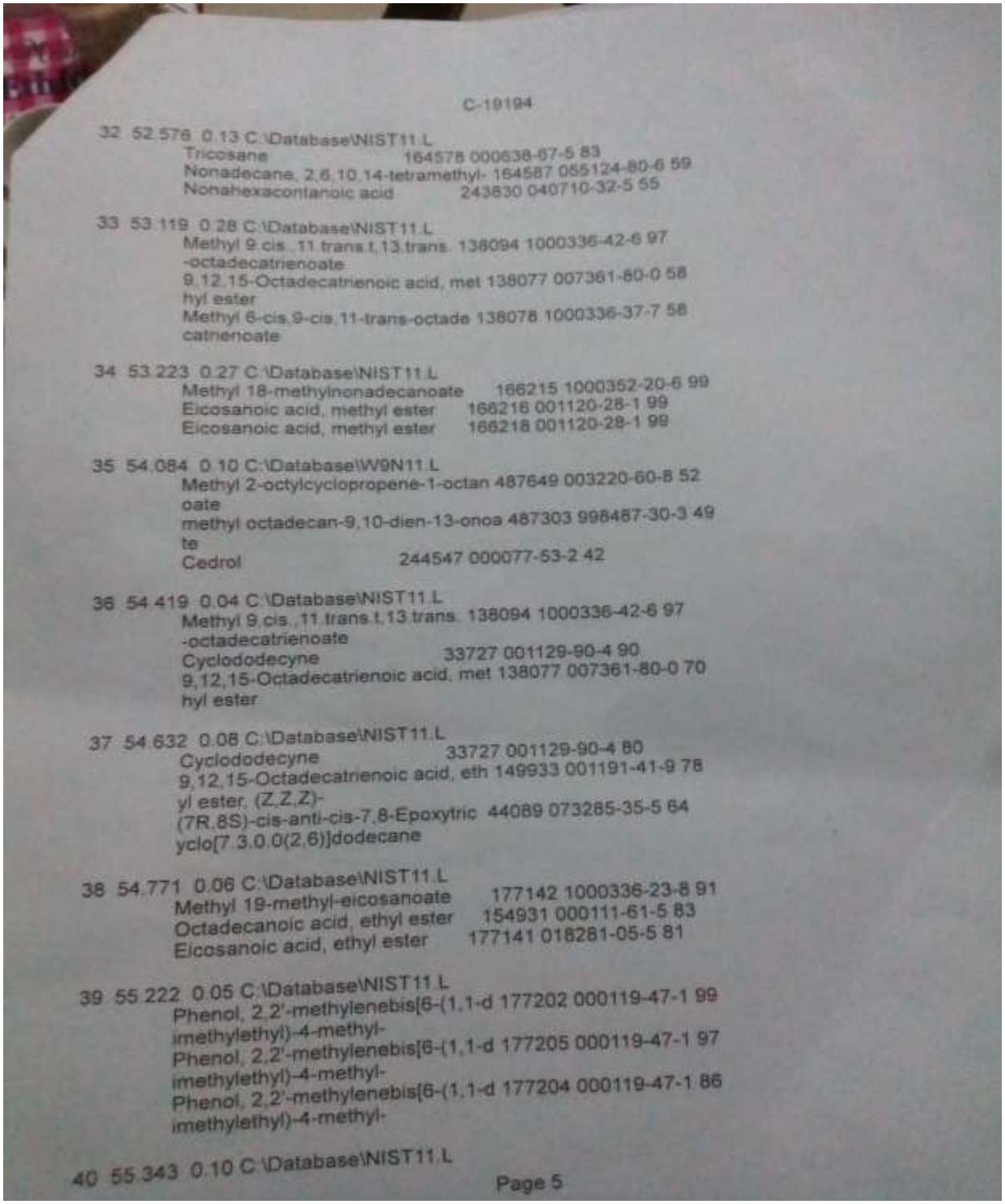
28 51.739 0.04 C:\Database\NIST11.L
9,12-Octadecadienoic acid (Z,Z)- 127647 000060-33-3 98
Methyl 10-trans,12-cis-octadecadie 139709 1000336-44-2 93
noate
9,12-Octadecadienoic acid, methyl 139729 002566-97-4 93
ester, (E,E)-

29 51.849 0.11 C:\Database\NIST11.L
Cyclohexaneethanol, 4-methyl- beta 26883 005502-99-8 87
-methylene-
12-Methyl-E,E-2,13-octadecadien-1- 127752 1000130-90-4 87
ol
E,E-10,12-Hexadecadien-1-ol acetat 127659 1000130-87-6 68

30 52.288 0.25 C:\Database\NIST11.L
9-Octadecenoic acid, 12-hydroxy-, 154825 000141-24-2 94
methyl ester, [R-(Z)]-
Methyl 12-hydroxy-9-octadecenoate 154792 1000336-28-8 87
9-Octadecenoic acid, 12-hydroxy-, 154816 127062-53-7 81
methyl ester, (Z)-

31 52.444 0.89 C:\Database\W9N11.L
Palmitoyl chloride $$ Hexadecanoyl 393487 000112-67-4 35
-chloride- $$ Palmitic acid chlori
de
Palmitoyl chloride $$ Hexadecanoyl 393489 000112-67-4 35
-chloride- $$ Palmitic acid chlori
de
o-Xylene-d10 14434 998014-43-4 30

Page 4



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Tricosane 164578 000638-67-5 83
Nonadecane, 2,6,10,14-tetramethyl- 164587 055124-80-6 59
Nonahexacontanoic acid 243830 040710-32-5 55

33 53.119 0.28 C:\Database\NIST11.L
Methyl 9.cis.,11 trans.t,13.trans. 138094 1000336-42-6 97
-octadecatrienoate
9,12,15-Octadecatrienoic acid, met 138077 007361-80-0 58
hyl ester
Methyl 6-cis,9-cis,11-trans-octade 138078 1000336-37-7 58
catrienoate

34 53.223 0.27 C:\Database\NIST11.L
Methyl 18-methylnonadecanoate 166215 1000352-20-6 99
Eicosanoic acid, methyl ester 166216 001120-28-1 99
Eicosanoic acid, methyl ester 166218 001120-28-1 99

35 54.084 0.10 C:\Database\W9N11.L
Methyl 2-octylcyclopropene-1-octan 487649 003220-60-8 52
oate
methyl octadecan-9,10-dien-13-onoa 487303 998487-30-3 49
te
Cedrol 244547 000077-53-2 42

36 54.419 0.04 C:\Database\NIST11.L
Methyl 9.cis.,11.trans.t,13.trans. 138094 1000336-42-6 97
-octadecatrienoate
Cyclododecyne 33727 001129-90-4 90
9,12,15-Octadecatrienoic acid, met 138077 007361-80-0 70
hyl ester

37 54.632 0.06 C:\Database\NIST11.L
Cyclododecyne 33727 001129-90-4 80
9,12,15-Octadecatrienoic acid, eth 149933 001191-41-9 78
yl ester, (Z,Z,Z)-
(7R,8S)-cis-anti-cis-7,8-Epoxytric 44089 073285-35-5 64
yclo[7.3.0.0(2,6)]dodecane

38 54.771 0.06 C:\Database\NIST11.L
Methyl 19-methyl-eicosanoate 177142 1000336-23-8 91
Octadecanoic acid, ethyl ester 154931 000111-61-5 83
Eicosanoic acid, ethyl ester 177141 018281-05-5 81

39 55.222 0.05 C:\Database\NIST11.L
Phenol, 2,2'-methylenebis[6-(1,1-d 177202 000119-47-1 99
imethylethyl)-4-methyl-
Phenol, 2,2'-methylenebis[6-(1,1-d 177205 000119-47-1 97
imethylethyl)-4-methyl-
Phenol, 2,2'-methylenebis[6-(1,1-d 177204 000119-47-1 86
imethylethyl)-4-methyl-

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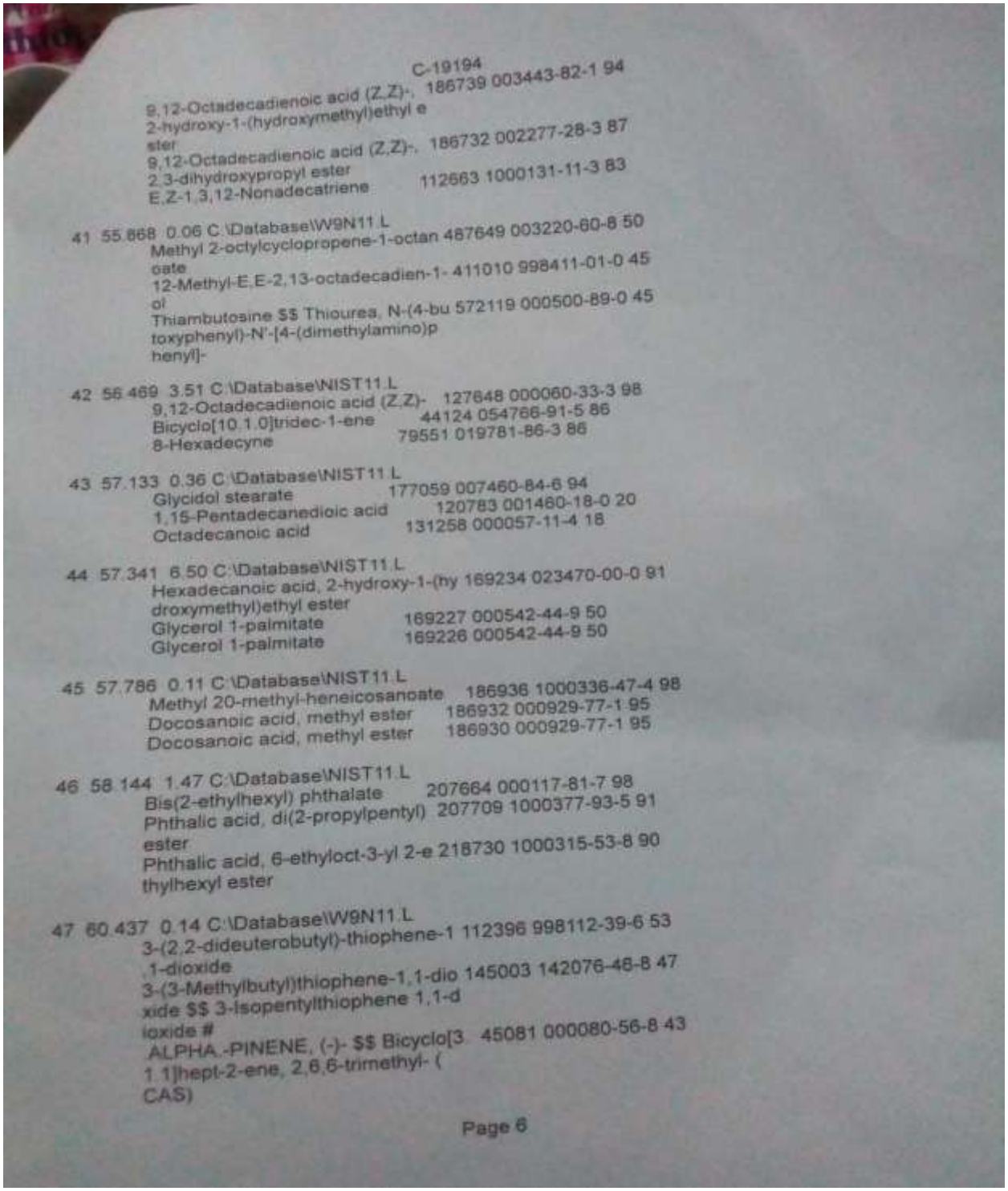
Page 5



C-19194
9,12-Octadecadienoic acid (Z,Z)-, 186739 003443-82-1 94
2-hydroxy-1-(hydroxymethyl)ethyl e
ster
9,12-Octadecadienoic acid (Z,Z)-, 186732 002277-28-3 87
2,3-dihydroxypropyl ester
E,Z-1,3,12-Nonadecatriene 112663 1000131-11-3 83

41 55.868 0.06 C:\Database\W9N11.L
Methyl 2-octylcyclopropene-1-octan 487649 003220-60-8 50
oate
12-Methyl-E,E-2,13-octadecadien-1- 411010 998411-01-0 45
ol
Thiambutosine $$ Thiourea, N-(4-bu 572119 000500-89-0 45
toxyphenyl)-N'-[4-(dimethylamino)p
henyl]-

42 56.469 3.51 C:\Database\NIST11.L
9,12-Octadecadienoic acid (Z,Z)- 127648 000060-33-3 98
Bicyclo[10.1.0]tridec-1-ene 44124 054766-91-5 86
8-Hexadecyne 79551 019781-86-3 86

43 57.133 0.36 C:\Database\NIST11.L
Glycidol stearate 177059 007460-84-6 94
1,15-Pentadecanedioic acid 120783 001460-18-0 20
Octadecanoic acid 131258 000057-11-4 18

44 57.341 6.50 C:\Database\NIST11.L
Hexadecanoic acid, 2-hydroxy-1-(hy 169234 023470-00-0 91
droxymethyl)ethyl ester
Glycerol 1-palmitate 169227 000542-44-9 50
Glycerol 1-palmitate 169226 000542-44-9 50

45 57.786 0.11 C:\Database\NIST11.L
Methyl 20-methyl-heneicosanoate 186936 1000336-47-4 98
Docosanoic acid, methyl ester 186932 000929-77-1 95
Docosanoic acid, methyl ester 186930 000929-77-1 95

46 58.144 1.47 C:\Database\NIST11.L
Bis(2-ethylhexyl) phthalate 207664 000117-81-7 98
Phthalic acid, di(2-propylpentyl) 207709 1000377-93-5 91
ester
Phthalic acid, 6-ethyloct-3-yl 2-e 218730 1000315-53-8 90
thylhexyl ester

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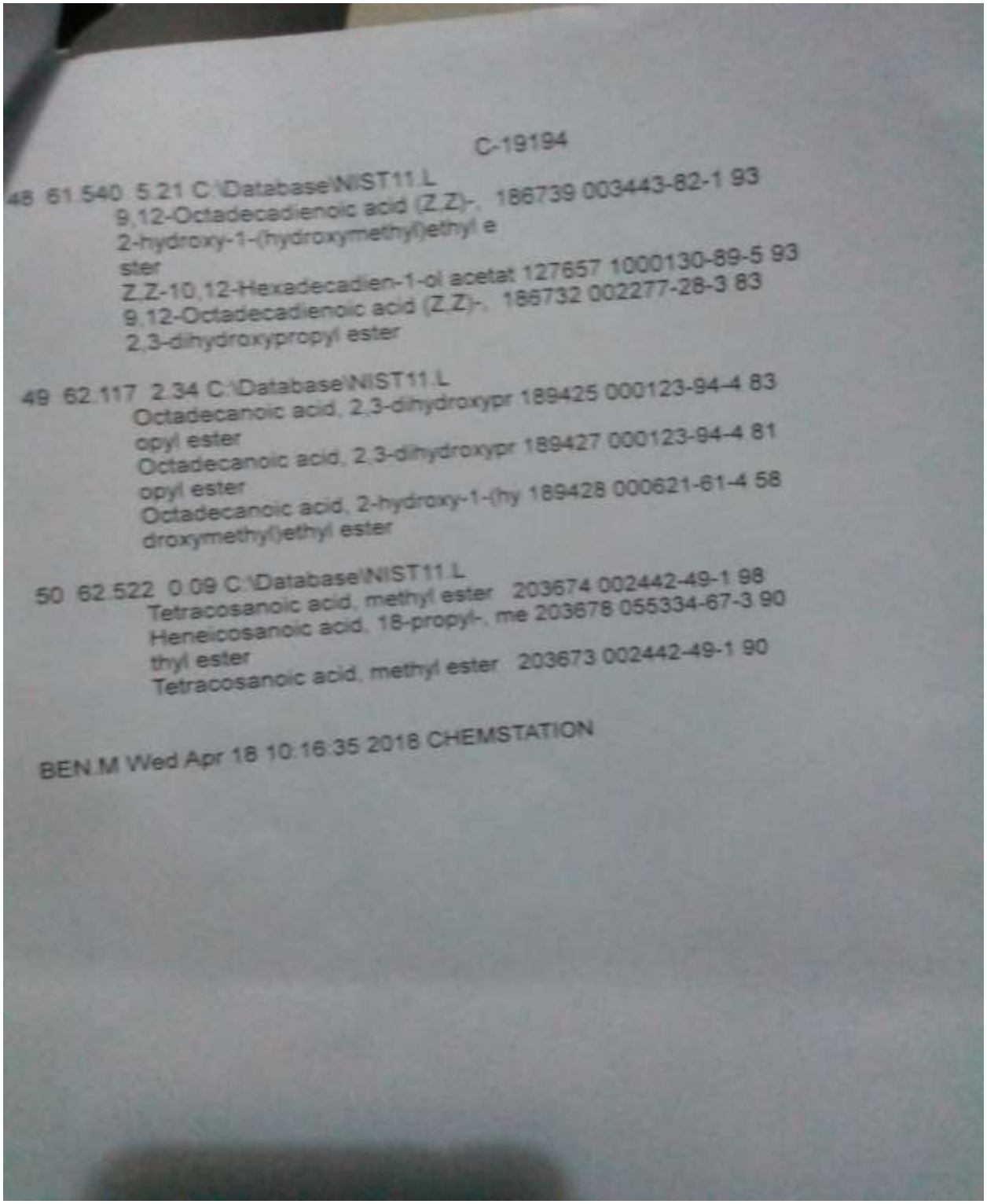
3-(2,2-dideuterobutyl)-thiophene-1 112396 998112-39-6 53
,1-dioxide
3-(3-Methylbutyl)thiophene-1,1-dio 145003 142076-48-8 47
xide $$ 3-Isopentylthiophene 1,1-d
ioxide #
ALPHA.-PINENE, (-)- $$ Bicyclo[3 45081 000080-56-8 43
1.1]hept-2-ene, 2,6,6-trimethyl- (
CAS)

Page 6



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48 61.540 5.21 C:\Database\NIST11.L
9,12-Octadecadienoic acid (Z,Z)-, 186739 003443-82-1 93
2-hydroxy-1-(hydroxymethyl)ethyl e
ster
Z,Z-10,12-Hexadecadien-1-ol acetat 127657 1000130-89-5 93
9,12-Octadecadienoic acid (Z,Z)-, 186732 002277-28-3 83
2,3-dihydroxypropyl ester

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Octadecanoic acid, 2,3-dihydroxypr 189425 000123-94-4 83
opyl ester
Octadecanoic acid, 2,3-dihydroxypr 189427 000123-94-4 81
opyl ester
Octadecanoic acid, 2-hydroxy-1-(hy 189428 000621-61-4 58
droxymethyl)ethyl ester

50 62.522 0.09 C:\Database\NIST11.L
Tetracosanoic acid, methyl ester 203674 002442-49-1 98
Heneicosanoic acid, 18-propyl-, me 203678 055334-67-3 90
thyl ester
Tetracosanoic acid, methyl ester 203673 002442-49-1 90

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Appendix: E. Laboratory equipment's and samples photo

Figure: E1. Matured pumpkin, half pumpkin, hulled pumpkin seed respectively

Figure: E2. peeled pumpkin seed and seive on the top view respectively

Figure: E3. Etracted pumpkin seed oil with ethanol in 500ml bottel , Extracted pumpkin seed oil on petri dish and extracted oil on using different parameter respectively

Figure: E4. Miller , seive vibirator set up and rotery evaporator respectively

Figure: E5. PH meter, spactrophotometer and 121 ^{0}c autoclave respectively

Figure: E7. Moller Hinton agar, molar Hinton agar on balance, sterilized molar Hinton agar respectively

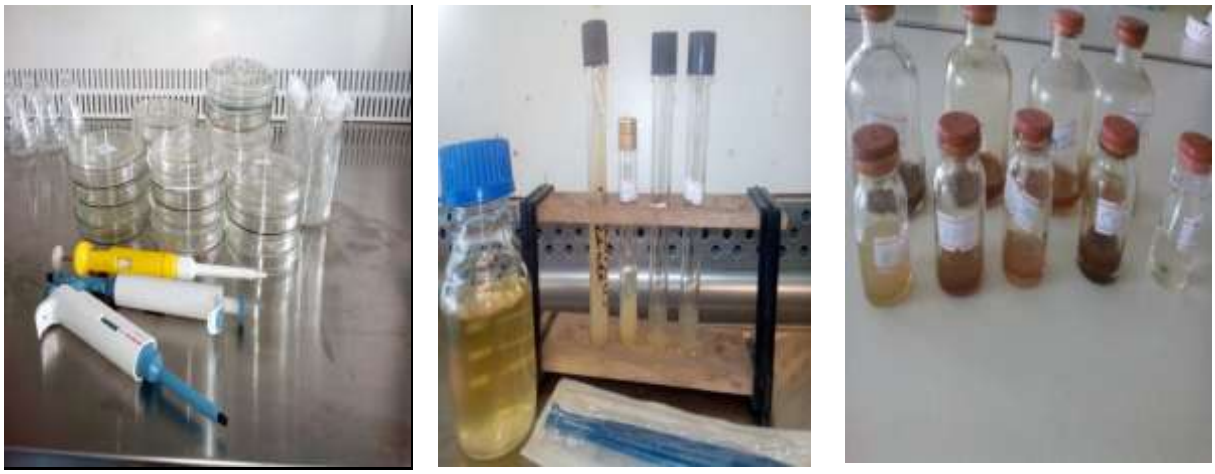

Figure: E8. Sterilized petir dish, sterilized molar Hinton broth and bacteria slant with their colonies and serial dilution of pumpkin seed oil in DMSO respectively

Figure: E9. Bio Hazard, 37^0c incubator and IKA* MS 3 digital shaker respectively