ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

Optimization and Characterization of Essential Oil from Black Pepper (Nigrum) seed and Leave Using Steam Distillation for Cosmetics Application Process

By Wondifraw abate

M.Sc. Thesis submitted to

The School of Chemical and Bio Engineering, Addis Ababa institute of technology (AAIT), Addis Ababa University

Presented in partial fulfillment of the requirements for the degree of Master of Science in (Chemical and Bio Engineering under Process Engineering stream)

Addis Ababa, Ethiopia

June 2018

Addis Ababa University
Addis Ababa Institute of Technology
School of Chemical and Bio Engineering

This is to certify that the thesis prepared by *Wondifraw Abate*, entitled: optimization and characterization of essential oil from black pepper seed and leaves using steam distillation for cosmetics application process and submitted in partial fulfilment 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:

Dr. Shegaw Ahmed _____ _____ _____

Advisor Signature Date

Dr. Anuradha Jabasingh _____ _____ _____

Internal Examiner Signature Date

Dr.Eng.Shimelis Adimassu _____ _____ _____

External Examiner Signature Date

_____ _____ _____

School or Center of Chair person Signature Date

DECLARATION

I declare that this thesis entitled optimization and characterization of essential oil from black pepper seed and leave using steam distillation for cosmetics application process 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 Information taken from published and unpublished work of others has been acknowledged in the text and a list of references is given.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.Shegaw.A* instructor in Addis Ababa University, School of Chemical and Bio Engineering.

Name: Wondifraw Abate

Signature: _____

Date of Submission: June 6, 2018

Abstract

The black pepper (piper nigrum) seed Oils were highly concentrate substances extracted from seed and leave. The study aims to utilize the seed as a source of oil for cosmetics skin care applications since piper nigrum seed and leave was cheap and grows abundantly in our country. It is an annual herbaceous plant. The characteristics of the seed are; the color is black after drying and the particle size for my experiment was 500,750,1000micro meters. The condition operation to extract of the seed and leave were 1atm and 30-250minute. The seeds produced colorless clear aromatic essential oil, used in the production of several licorous cosmetics and of perfumery. The seed were extracted by steam distillation because it had high conversion efficiency. The steam distillation were carry out under controlled temperature and pressure. The optimum temperatures of the extraction 105 ^{o}c,127.5 ^{o}c,150 ^{o}c. the yield of the oil was 4.83% at the optimum conditions and the minimum yield 1.67%.The characteristic of piper nigrum seed was strong pleasant aroma. These oils were often used for their cosmetics and their therapeutic or odoriferous properties, in a wide selection of products such as foods and medicines. Cosmetics oil was one of the most time- and effort consuming processes. Cosmetic composition mainly includes pigment, fatty acids, oleic acid 22.94%, and hexandoxic acids octandoxic acid 8.61%. The anti-bacterial properties of the oils were 60% greater efficient. The acid value of the oil 5.3 by using NaOH titration with ethanol solution. The aroma odor were changed into good odor by addition of Lemmon oil. Cosmetic products had homogeneous and stable during the application.

There had wide number of ways to extracted the oil but the quality never remains the same. Here I used the "Steam Distillation" method for extraction which was the cheapest way for the extraction of Oils and the efficient from the different parts of the plants. In this process steam was allow to pass through the extraction chamber which contains plant matter. When steam passes through the seed material under pressure which often the cells and allows the oil to escape in vapor form. The vapor allows to pass through condenser and oil was collected in separating tank and store in storage tank. The analysis of cosmetics oil reveal that parameters, which include specific gravity, FTIR, GC_MS, acid value, for skin oil process.

Keywords: piper nigrum seed, cosmetics, yield, oleic acid, Steam distillation.

ACKNOWLEDGEMENTS

Firstly, I would like to thank the Almighty GOD for giving me the strength and wisdom to successfully complete this thesis for his protection and strength, and an ever present helps in the entire situation and challenge that I face.

I express sincere gratitude to my Thesis guide Dr. Shegaw Ahmed, for his kind support, guidance and constructive criticism in the completion of my research. I would like to say thankful to Dr. Beteley .T, for his sincerity over the research.

I would also like to thank my good friends, Mr. Yemane.G, Mr. Tlahun.G and Mr. Kitaw Abraham, for sharing what I know and for lending me some materials and books to be used as reference.

I also would like to express my gratitude to the laboratory technicians of the chemical engineering department in general and to Ato Hintsasilase Sefu in particular for their time and help during my experimental work; and Leather Industry Development Institute staffs Ms.Meron and Mr. Hayilemariam for providing me the necessary opportunities for the completion of laboratory analysis of my Thesis.

TABLE CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS

AO	amount of raw materials introduced to distillation unit
ANOVA	analysis of variance
AS	amount of substance or oils after extractions
CO	cosmetics oils
DF	degree of freedom
PEO	pepper essential oils
PSO	pepper seed oils
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	gas chromatography and mass spectroscopy
MNUFA	mono unsaturated fatty acids
NAM	nutrient agar media
OA	oleic acids
OW	oil in water
SG	specific gravity
STD	standard order
TSST	toxic shock syndrome toxin
USFA	unsaturated fatty acid

1. INTRODUCTION

1.1 Back ground

Ethiopia is a potential country in resourceful for essential oils in the producing cosmetics. "Essential oil" or "etheric oil" means a volatile oil and is obtained from plants and animal body by different methods the essential oil which is getting from spices, such as; pepper oil, cinnamon oil, lemon leaves, clove oil, fennel, black pepper, orange, ginger, etc. pepper seed oil is commonly used as a medicine, pharmacies, condiment, cosmetics and perfumes. This oils are volatile, fragrant and pleasant tasting oils obtained from seed and leave. It has great applications in pharmaceutical, foods, perfumery and cosmetics. A variety of plants have a high content of oils that are feasible. These plants are locally crop plants, requiring little maintenance and grow in almost all parts of our country (Damayanti & Setyawan, 2012b)].

It was known as *madhurika* and its cultivation in India is thought to date back at least to 2000 BC. it was also a symbol of success to the Romans and pepper leaves were used to crown victors in games (Ministry, Welfare, & View, 2016).

Cosmetic composition mainly includes pigment, fatty binder, and filler. Cosmetic products must be homogeneous and stable during the application. Skin disease is common ailments of all age groups because of the infection of a variety of microorganism, chemical agents and biological toxin present in the atmosphere and also due to physical factors, malnutrition and environmental pollution. Similar Fatty acids are natural components of skin and are components of a complex that makes up the outermost layer that protects the body against oxidative damage. The extraction of oil from these varieties poses no special problems and the end product is marketable both locally and abroad. Pepper seed, is an excellent source of cosmetics oil. It grows abundantly in our country. Which is the chief constituent of cosmetics oil the physical properties of the seed, like those of other grains and seeds, are essential for the cosmetics application for hair food, skin care, and processing or determining the behavior of the product(Mohammedi & Aminifard, 2013).

1.2 PROBLEM STATEMENT

Essential oils are one of the world leading compounds that are produce from different raw materials for the purpose of medicines, perfumes, cosmetics etc. in our country a lot of essential oils are imported from different countries. One of the application of essential oil is to use for cosmetics (skin care). This oils have high price and costly due to the case of production processes and raw materials availability. For this reason, there was no interest and enough study carried out to extract from local raw materials as a source of essential oil for the application of cosmetics. So as to minimize this problem, to substitute the raw materials of essential oil by local crop pepper seed and leave. Thus to add some values for community by local substitution of essential oils, it is very essential to explore the abundant material for its potential for cosmetics.

In general, Ethiopia imports essential oils. It has potential raw materials to produce essential oil. Due to the incredible versatility of the product which could be produce in many different areas depending on what raw material is available to get high yields. Therefore, this study were conducted to investigate the potential of the locally available crop product namely pepper seeds as a new raw material for cosmetics application. In recent research, from literature survey and as our knowledge the extraction and characterization of pepper seeds were not the first. Previously, it also used for pharmacicual application for insecticide and traditionally human being used as drier of injured body.

The seeds were available in all regions of Ethiopia and can grow abundantly. It is tropical zone annual plant, mostly available in semi dry farming land areas. What makes interesting about the seed oil is that the oil more than 30 types of terpene compounds in the essential oil of nigrum, the most important of them are 50 to 80% fatty acids, 8% fenshon and limonene 5%. This herb also contains phenolic compounds such as flavonoids, phenolic acids, hydroxyl cinnamic acids, coumarin and tannin. Phenolic acids include 3-O-Caffeoylquinic acid, contains as the major UN saturated fatty acid. Oil obtained from the nigrum seed has 4% palmitic acid, 22% oleic acid, 14% linoleic acid and 6% petrocylic acid. The seed oil has value of 4 to 6% essence which its essence and combine ingredients vary according to the location of plant growth (Rios et al. 2011). The pepper fatty acid has unique characteristics for application of skin care cosmetics through different processes and has low viscosity.

The extraction and the characterization of the nigrum seeds affected by various factors. One of the factor was temperatures, the high value of the temperature were not allowed for the extraction and characterization. However the extraction of essential oils from this materials has different minerals, unsaturated fatty acids, mono unsaturated fatty acids, polyphenols, aromatics and alcohols. The essential oil of this has several draw backs such as aromatic odor, anti -oxidant. In addition of this the seed has not well known for the skin care cosmetics application.

1.3 OBJECTIVES OF THE RESEARCH

1.3.1 General Objective

Generally the objectives of this study relies on optimization and characterization of essential oil extracted from black pepper seed and leave using steam distillation extraction process for cosmetics application.

1.3.2 Specific Objective

This study was specifically:

- ✓ To determine the proximate composition of the black pepper seeds.
- ✓ To investigate the effect of temperature, time, particle size on the yield of pepper oil and perform optimum condition of the extraction to get high yield of essential oil.
- ✓ To characterize the physico-chemical and anti-bacterial properties of extracted oil.
- ✓ To evaluate the extracted oil for application of cosmetics.

1.4 SIGINIFICANCE OF THE STUDY

This research has many benefits for stallholders in the economy:

❖ Provided experimental results about the main ingredients of local seed oils as source essential oils for cosmetics and also assess the mineral concentration.

❖ To made interest the used of this oils as cosmetics application for customers and institutions.

❖ Provided baseline information for stakeholder institutions and farmers involve in crop of seeds.

❖ Performed as a piece of evidence for further research in technology and quality analysis.

2. LITERATURE REVEIEW

2.1 Extraction of Essential Oil and Mechanism of Extraction

Essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. The world production and consumption of essential oils and perfumes are increasing very fast(Hassan, Hamad, 2009).Essential oils are intense volatile aromatic compounds produced by plants the easily evaporated gist's that give plants their wonderful scents. Each of these complex precious liquids is extracted from a particular species of plant life. Each plant species originates in certain regions of the world, with particular environmental conditions and neighboring fauna and flora. Essential oils are frequently referred to as the "life force" of plants. Unlike fatty oils, these "essential" oils are volatile, highly concentrated, substances extracted from flowers, leaves, stems, roots, seeds, bark, resin or fruit rinds. The amount of essential oils found in these plants can be anywhere from 0.01 percent to 10 percent of the total.(Liu, 2009). That's why tons of plant material are required for just a few hundred pounds of oil. These oils have potent antimicrobial factors, having wide range of therapeutic constituents. These oils are often used for their flavor and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics. Beware of imitations. Essential oils cannot be substituted with synthetics. Only pure oils contain a full spectrum of compounds that cheap imitations simply cannot duplicate(Dreger & Wielgus, 2013).Essential oils have vast applications for personal care, including beautifying the skin and hair, cleansing the mouth, gums, and teeth, and other uses for general hygiene. Because each essential oil has a unique chemical profile that provides different benefits, it is easy to use oils for a wide variety of tasks that relate to personal hygiene. Since ancient times, essential oils have been used to promote healthy skin, a clean mouth, strong fingernails and toenails, shiny hair, and more. Not only do natural cleansing, soothing, and purifying properties make essential oils an ideal choice for personal care, but the user can simultaneously enjoy the lovely, inviting smells of essential oils during use(Rozalli, , 2014).

2.1.1 Sources of Natural Essential Oil

Essential oil" or "etheric oil" means a volatile oil and is obtained from plants and animals by different method. Essential oil is the basic material of producing perfumes and cosmetics. Essential oils are generally originate from one or more plant parts(Metabolites, 2011).The essential oil which is obtained from spices, such as flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary,), leaves (e.g. fennel mint, lemongrass), leaves and stems (e.g. geranium, patchouli, verbena, cinnamon), bark (e.g. cinnamon, cassia, canella), wood (e.g. cedar, sandal, pine), roots (e.g. angelica, sassafras, vetiver, valerian), seeds (e. g fennel, coriander, pepper caraway, dill, nutmeg), fruits (bergamot, orange, lemon, juniper), rhizomes (e.g. ginger, curcuma) and *pepper* belongs to nigrum plant taxonomic species(Q & Flora, n.d. 2009).

2.1.2. Extraction Methods of Natural Essential Oils

The choice of method for extracting the essential oil depends on the original state and the characteristics of the plant raw material. The ratio of essential oil to plant raw material can be highly variable depending on the plants and can range from 0.015 % to over 20 %.(Afiq, Rahman, & Man, 2013). The extraction method determines characteristics of the essential oil such as viscosity, colour, solubility, volatility, and can cause enrichment or depletion of some components. Some extraction methods described below.

2.1.2.1. Steam Distillation

The essential oil is produced by the passage of steam through the plant raw material in a suitable apparatus. The steam may be introduced from an external source or generated by boiling water below the raw material or by boiling water in which the raw material is immersed. The water and essential oil are then separated by decantation(Hans, 2011).

2.1.2.2 Dry Distillation

The essential oil is produced by high-temperature heating of stems or barks in a suitable apparatus without the addition of water or steam(Berk & Zielke, 2013). It is the heating of solid materials to produce gaseous products (which may condense into liquids or solids). The product are condensed and collected.

2.1.2.3 Hydro-Distillation

Hydro-distillation (HD) is a variant of steam distillation, Instead of the steam input, the plant materials in HD are directly immersed in water. This solid-liquid mixture is then heated until boiling under atmospheric pressure in an alembic, where the heat allows the release of odorous molecules in plant cells. In order to isolate essential oils by hydro distillation, the aromatic plant material is packed in a still and sufficient quantity of water is added and brought to a boil, alternatively, live steam is injected in to the plant charge. Due to the influence of hot water and steam, the essential oil is separated from the oil glands in the plant tissue(Rooti,etal.,2011).

2.1.2.4. Mechanical Process

The essential oil is produced by a mechanical process without any heating. It is mainly applied to Citrus fruits, orange, and lemon.it involves the expression of the oil from the pericarp and subsequent separation by physical means. The conventional method of 'cold-pressing' requires an abrasive action to be applied to the entire surface of the fruit under a stream of water. After removal of solid waste, the essential oil is separated from the aqueous phase by centrifugation. Most industrial facilities actually allow simultaneous or sequential production of fruit juice and essential oil(Q & Flora, n.d.1999).

2.1.2.5 Supercritical Fluid Extraction

It is the process of separating one component (the extracting) from another (the matrix) using supercritical fluids as the extracting solvent. Carbon dioxide is the most commonly used supercritical fluid. A supercritical fluid is any substance at a temperature and pressure.

2.2 Black Pepper (nigrum) Seed Essential oils

Nigrum essential oils (NEO) are aromatic oily products. Also known as volatile or ethereal oils, these oils from the fixed or glyceride plant, seed oils, and the mineral oils. Nigrum oils have been obtained from 90 plant families, and at times different cosmetics oils can be secured from different parts of the same plants. Cosmetics oils (CO) are mixtures of organic chemicals formed as by-products of plant metabolism(Coelho et al., 2012).Their principal constituents are polyphenols, terpenes, but aliphatic and benzenoid components may also be present. This oils vary in properties

according to plant species, climate, soil, time, production and storage processes. Nigrum oil had been fatty acids oil contains for about 50-60%. The oil quality is determined by the amount of fatty acids content. The highest of seed oil content is from is 6.15%(Damayanti & Setyawan, 2012a) .nigrum seed oil (NSO) is necessary ingredient in the manufacture of cosmetics, condiment, perfumes and other products. At present, our country in large quantities is importing different type of cosmetics oil for the purpose of pharmacicual, condiment cosmetics. nigrum is found to be native in our country but it is not still usable for the purpose of oil, and used for condiment and other traditional medicinal purposes only(Shin, etal., 2007). It grows abundantly in all parts of region and is now being local cultivated for condiment. It has been valued for its numerous different uses. Its oil is used as cosmetics for skin care and hair food, in medicinal purpose, in the perfume industry, and in the scenting of soaps, detergents shampoos, lotions and others (Ashish, 2014).when we see the piper nigrum leave some little difference the amount of the composition and the ingredient the oleic and linoleic acid content.

2.2.1 Properties of Nigrum Seed

2.2.1.1 Physical and Chemical Properties of the Seed

The seeds are brown or green in color when freshly cut from the plant and turn slowly to a dull grey as the seed set for distinct period. Green seeds are best for cooking. Nigrum seeds are well known for their distinctive pleasant and are thus used for cream cosmetics. Dried nigrum seed is an aromatic, anise-flavor spice(Kacar, & Goksu, 2013). The chemical composition of pepper varies with source, climate and harvesting stage. pepper are one of the highest plant sources of potassium, sodium, phosphorus, and calcium, nigrum are richest in dietary fiber and vitamins, relative to human needs. They have smaller amounts of many other nutrient Every 100 g edible portion of its seeds contain on average: 8.8 g water; 15.8 g protein; 14.9 g fat; 36.6 g carbohydrate; 15.7 g fiber; and 8.2 g ash (containing 1.2 g Ca, 19 mg Fe, 1.7 g K, 385 mg Mg, 88 mg Na, 487 mg P and 28 mg Zn)(Gulfraz, Mehmood, Minhas, Jabeen, & Kausar, 2015). Every 100 g contains: vitamin A; niacin (6 mg); thiamine (0.41 mg); and riboflavin (0.35 mg); with an energy value of about 1440 kJ. The seeds contain mucilage, sugars, starch, tannin, essential oil and fixed oil (the main components of the fixed oil being petro selenic, oleic, linoleic and palmitic acids (Bernath et al., 1994)). The variety and quantity of vitamins contained is variable: folates, 270 mg/ kg; vitamin

B3, 6.4 mg/kg; vitamin C, 8.7–340 mg/kg. its contains potassium (4.24–5.85 g/kg), the most abundant mineral by far, with low amounts of phosphorus (500 mg/kg), calcium (5.6–363 mg/kg), magnesium (8.2–389 mg/k) and sodium (7.7– 512 mg/kg)(Ministry, Welfare, Food, et al., 2016). The color of the leaves the same to its seeds. When we saw the chemical properties in some extent some difference. Its leaves contain vitamins and minerals such as calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin c. It consist 10 to 12 % of oil that is stored in the cotyledons of seeds. nigrum leave oils contains 6.3% water, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fibers and 42.3% carbohydrates(Afiq et al., 2013).

2.3 Extraction Process of the Nigrum oil

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(Pyrophosphate, n.d.). 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)(Berk & Zielke, 2013). 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°C, at atmospheric pressure. its seeds were dried at 105^{o}C in hot air oven till constant weight is attained(Mahajan, & Dua, 2013).

Fresh, or sometimes dried, botanical material is placed in the plant chamber of the still and the steam is allows to pass through the herb material under pressure which softens the cells and allows the Essential Oil to escape in vapor form. The best particle size of raw material for extraction of essential oil between 0.5mm and 2.5mm (Rozalli et al., 2014). the particle size of a powder material is expressed is usually defined by the method by which it is determined a sieve analysis are used(Liu, 2009).

The temperature of the steam must be high enough to vaporize the oil present, yet not so high that it destroys the plants or burns the Essential Oils. Besides the steam tiny droplets of Essential Oil evaporates and travel through a tube into the still's condensation chamber. Here Essential Oil vapors condense with the steam(Vivek, 2012). The essential oil forms a film on the surface of the water. To separate the Essential Oil from the water, the film is then decanted or skimmed off the

top. The remaining water, a byproduct of distillation, is called floral water, distillate, or hydrosol. It retains many of the therapeutic properties of the plant, making it valuable in skin care for facial mists and toners A solution containing chemicals that can change the color of a photographic print(Sarkic & Stappen, 2018).

The optimum operation of essential oil extraction is:

- Solvent concentration: 0 – 90 % ethanol;
- The mixture of ethanol to water ratio1:3
- solid -to- Liquid ratio: 5 – 20;
- Temperature: 100–200 °C;
- Time: 30 –250 minute.

The extraction yield highly dependent on the solvent polarity. The highest yields are usually achieved with ethanol and methanol, and their mixtures with water, although other solvents, such as ethyl acetate or acetone, have also been used for extraction of polyphenols from plants(Part et al., n.d.). Water and ethanol are the most widely used because of their low toxicity and high extraction yields. The yield of the nigrum oil around 6%.-6.12% and above greater based on the solvent equipment consideration. the essential oil content increased with increase of DT and reached a maximum of 0.78% at 160 min DT(Kooti et al., 2015). These solvents are suitable for extraction of phenolic compounds, most of which are also of polar type. Additionally, the resulting polarity of water-ethanol mixtures varies depending on the proportion of solvent constituents, In order to determine the concentration evolution in the course of time, standard deviations will be select(Hans, 2011).

$$C(t) = Co + \frac{t}{K1t+K2} \quad \text{..... eqn (2.1)}$$

C (*t*) is the concentration of extracted substance after time *t*, *K*1 and *K*2 are constants, *C*o is the concentration of extracted substance at the initial time. This can be reduced to:

$$K1t + K2 = t/C(t) \quad \text{.....eqn (2.2)}$$

$k_1$and K_2 determine from slop of line. (Angelov & Boyadzhieva, 2016).

2.4 Properties of the Seed Oils

2.4.1 Physical and Chemical properties

black pepper(nigrum) seed oils are generally colorless to slightly yellowish when freshly distill, but when foreign matter is present, the color may range from red to blue(Unal et al., 2013). On standing, the oils generally become darker in color. The odor of seed oils is similar to that of the portion of the plant from which they are derived and these odorous characteristics are much more concentrated in the cosmetics oil.

The SG of these materials varies from 0.84-1.18. Solubility in alcohol 90% perfectly soluble. They are volatile to room temperature and evaporate when heated. Most oils are slightly soluble in water and they are more soluble in sugar solutions(Patel, & Bandivdekar, 2014). *Nigrum* seed oil has fatty acids, phenolic components, hydrocarbons, volatile components, and few other classes of secondary metabolites. Aqueous extract of Nigrum seeds are rich in phenolic compounds. The extract of the seeds contains rosmarinic acid, chlorogenic acids as major phenolic compounds (14.9% and 6.8%, resp.), and quercetin and apigenin as the major flavonoids (17.1% and 12.5%, resp.). Also, the total phenolic compounds in Nigrum were higher than the flavonoid compounds.(Brazil, 2006). Carbohydrates are the most abundant macronutrients in all the parts and range from 18.44 to 22.82 g/100 g. Proteins, reducing sugars, and fats are the less abundant macronutrients; proteins varied between 1.08 g/100 g in stems and stems revealed the highest fat content (1.28 g/100 g) and reducing sugar content (1.49 g/100 g), respectively, amongst all the parts of black pepper. On the basis of the proximate analysis, it can be calculated that a fresh portion of 100 g of these parts yields, fatty acids present in all the parts. Monounsaturated fatty acids (MUFA) as the main group of fatty acids. Nevertheless, unsaturated fatty acids (UFA) range from 66% to 80% and predominate over saturated fatty acids. The highest concentration of n-3 fatty acids was found in black pepper leaves (Badgujar et al., 2014).

2.4.2 Nigrum Seed oil of Phytochemical Compositions

All parts of black pepper such as roots, leaves, fruit and especially the seeds oil are used for medicine, cosmetics, flavors, condiment etc. Oil obtained from the seed has 4% palmitic acid, 22% oleic acid, 14% linoleic acid and 6% petrocylic acid. The seed oil has value of 4 to 6% essence

which its essence and combine ingredients vary according to the location of plant growth(Rios , 2011). The aromatic property of nigrum is because of the essence. There are more than 30 types of terpene compounds in the essential oil of black pepper, the most important of them are 50 to 80% fatty acids, 8% fenshon and limonene 5%. This herb also contains phenolic compounds such as flavonoids, phenolic acids, hydroxyl cinnamic acids, coumarin and tannin. Phenolic acids include 3-O-Caffeoylquinic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, 1, 3-O-di-caffeoylquinic acid, 1, 4-O-di-caffeoylquinic acid and 1, 5-O-di-caffeoylquinicacid. Its flavonoid contains eriodictyol-7-rutinoside, quercetin-3-rutinoside and rosmarinic acid. Also aqueous extract of fennel seed include quercetin-3-O-galactoside, kaempferol-3-O- rutinoside, kaempferol-3-O-glucoside, quercetin-3-O- glucuronide, kampferol-3-O-glucuronide,isoquercetin, and isorhamnetin-3-O-glucoside(Kooti et al., 2015). The total phenolic and flavonoid contents of wild fennel (2.4% and 1.2% resp.) were less as compared to cultivated fennel (3.1% and 1.6%, resp.)(Badgujar et al., 2014).

2.4.3. Anti -bacterial Properties of the nigrum oil

The oil has been used as an ethnic rectify for the cure of numerous infectious disorders of bacterial, fungal, viral, and mycobacterial origin. Several studies have been carried out in the past validating its antimicrobial, anti-mycobacterial, and antiviral potential properties. The antibacterial effect of the aqueous extract of nigrum plants have so money application. An aqueous extract of the aerial part of the inhibited the growth of Agrobacterium radio bacteria, and Pseudomonas glycinea. An aqueous extract of seed sample inhibited the growth of Enterococcus faecalis, Staphylococcus aureus, Escherichia etc.

Antimicrobial activity of the essential oils results indicated that all essential oil samples have antibacterial activity against Gram negative and Gram positive bacteria. The most effective oil against Gram negative bacteria was Nigrum, which is less effective than ampicillin by 25% and 7% in the Escherichia coli and Pseudomonas aeruginosa bioassays, respectively, while the most effective essential oil against Gram positive bacteria was nigrum which gave a larger inhibition zone than ampicillin by 58.3% and 114% in the Staphylococcus aureus and Bacillus subtilis tests, respectively(Composition & Cultivars, 2011).

Essential oil of F. had significant antimicrobial activities against some of microorganisms as compared to the methanol and ethanoic extracts. The diameters of growth inhibition zone ranged

from14 to 31mm (including the diameter of the disc 6mm) with the highest inhibition zone values observed. Nigrum inhibited the growth of Staphylococcus aureus and Bacillus pumilus with 11.27 and 12.67mm zone of inhibition, respectively. All bacterial cultures were maintained and sub cultured regularly on Nutrient agar media (NAM) containing peptone 5 g; beef extract 3 g; sodium chloride 5 g and agar 2% in a final volume of 1l. The size of inoculum was adjusted to approximately 108 colony-forming units per ml by suspending the culture in sterile distilled water. Petri dishes containing nearly 25 ml of nutrient agar medium were seeded with 100 µl culture of the respective bacterial strains and kept for 15 min for the absorption of culture(Dua et al., 2013). The skin and mucous membrane are excellent barriers against local tissue invasion. However, if either of these is breached due to trauma or surgery, S. aureus can enter the underlying tissue, creating its characteristic local abscess lesion (Elek, 1956), and if it reaches the lymphatic channels or blood can cause septicemia (Wald Vogel, 1990). The basic skin lesson caused by an S. aureus infection is a pyogenic abscess. However, S. aureus can also produce a range of extracellular toxins, such as enterotoxin A-E, toxic shock syndrome toxin-1 (TSST-1) and exfoliative toxins A and B (Projan and Novick, 1997).

The mechanism of action of essential oils may be related to their lipophilic character. They are well absorbed by the nasal, oral, gastric, intestinal mycosis membranes and skin. In large concentrations they may have irritant activity. The active compounds contained in the oils are incorporated into cell membranes and influence enzyme and ion channel function and receptor proteins(English, Manager, & Products, n.d.). Oils do not accumulated in the human body and are neutralized by binding to glucuronic acid and eliminated with urine. Essential oils demonstrate bactericidal activity against both Gram-positive bacteria as well as however, lover due to complex structure of cell walls against Gram-negative bacteria with the range from 50 to 1000 mg/ml and even at 0.25-1.0 ml amounts for most active oils(Sienkiewicz, Denys, & Kowalczyk, 2015). Antimicrobial efficacy of essential oils against bacteria, fungi, protozoa and viruses is strictly linked to their chemical composition. Bactericidal properties depend on the lipophilic character of hydrocarbon skeleton and hydrophilic character of functional groups. The highest antibacterial activity and the widest spectrum of action are demonstrated by phenolic compounds(Sienkiewicz et al., 2015).

2.4.4 Bacterial Growth and culturing media

To grow bacteria in the lab, environmental conditions, as well as nutrients, must be considered. Bacteria may be isolated from a variety of environments. For cultivation of bacteria in the lab, the conditions of the environments must be mimicked. Prokaryotes that live in extreme environments are generally in the Domain: Those that live in more moderate environments are generally in the Domain: Bacteria. Consider temperature.

Microorganisms whose optimal temperature for growth is near 0^oC are called psychrophilic. Those that grow optimally between 20^oC and 45^oC are called mesophilic.

Those growing best at high temperatures, 55^oC to ~ 70^oC and higher, are called thermophiles. Prokaryotes that live at very high temperatures (up to 113^oC) are hyperthermophiles. Consider salt tolerance. Some bacteria require relatively high concentrations of salt for growth (10-20%); these organisms are called halophiles. Halophiles must possess special membranes and enzyme mechanisms to survive in salty environments. Some organisms are salt tolerant. They do not require salt for growth but may grow in its presence. An example is *Staphylococcus aureus. S. aureus* is found on skin, which often has a high salt concentration (10% NaCl). Consider the requirement for oxygen. An organism that *requires* oxygen for growth is called aerobic.

S. aureus is a mesophilic organism with optimum growth temperature in the range from 37 °C to 40 °C. The minimal temperature for growth is about 7.0 °C, but some strains do not even show growth at 8 °C. On the other hand, a temperature higher than 46 °C is not acceptable for the majority of strains, with some exceptions that do grow up to 50 °C(Shin et al., 2007). Heating causes damage to the cell. In human being the species *Staphylococcus aureus* was found Gram-positive, non-motile, cocci in shape. The cells of this species were arranged in singles, pairs or in short chains. Cultural characteristics on nutrient agar, the species produced yellowish, golden and shiny colonies(Dua et al., 2013)**.**

2.4.5 Procedure for Preparation of Nutrient Agar and Serial Dilution

Bacterial cells were very small and could be difficult to count from a slide without magnifier. They also grow to a high density in a liquid culture, which could also make counting them rather tricky. When a bacterial culture in liquid media was introduced to a nutrient agar plate, each

individual viable bacterium was form a colony, which were visible to the eye (28gm nutrient agar measured for 1000ml distilled water and sterilize, this provided the nutrients this bacterium needs). The colonies were then be counted and you would then be able to extrapolate the number of viable bacteria in the entire culture. The following materials listed below:

11 agar petri dish 3 sterile swabs 2 sterile transfer pipette

100 ml sterile nutrient agar 11 sterile tubes

1. Prepared the 11 tubes and fill 9ml distilled water into for 10 tubes the other one tubes must be 10 ml distilled water and added ml .
2. Using the transfer pipette, fill each of the 10 empty sterile tubes with 9 mL of sterile media. Be careful not to touch the sides of the tubes or the table with the pipette.
3. Withdraw 1 mL of culture staphylococcus aureus. Added to the tube labeled 1/10. Mixed well by swishing the solution in and out of the pipette. Withdraw 1mL of this solution. Added to the tube labeled 1/100 etc. for the same procedure the other 9ml tubes fill with took sequential process mean that after dilution withdraw 1ml from first tube into the second tube then after from second tube I took 1ml into the 3^{rd} tube then withdraw 1ml sequentially until 11^{th} tube would become fill.
4. Dip a sterile swab into the tube labeled 1/10, press it against the side of the tube to squeeze out excess liquid, gently rub the wet swab over the surface of the agar petri dish labeled 1/10 dilution.
5. Dip a sterile swab into the tube labeled 1/100, press it against the side of the tube to squeeze out excess liquid, gently rub the swab over the surface of the agar petri dish labeled 1/100 dilution. The same procedure for others tubes were applied.
6. Discarded the tubes containing my dilutions.
7. Incubated the petri dish for 24 hours, then counted and recorded the number of colonies on each petri dish.
8. Calculated the number of bacteria from the petri dish cultured using the formula:

Number of bacteria = number of colonies/dilution factor

The following tables were to conclude bacterial culturing process.

No. sample	1	2	3	4	5	6	7	8	9	10	11
Sterile tubes amount	10ml	9ml	9ml	9ml	9ml	9ml	9ml	9ml	9ml	9ml	9ml
Serial dilution		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}

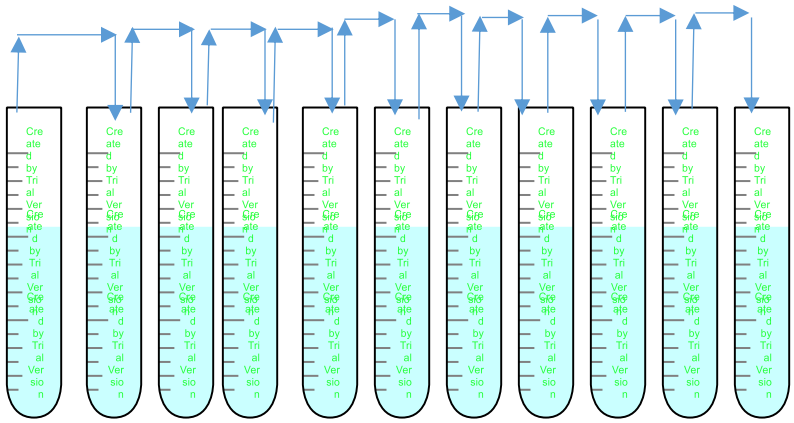


Figure 2.1 serial dilution addition process

2.5 Benefits of Black Pepper (nigrum) Seed oils

There are other important ways in which piper nigrum serves as a therapeutic agent, relaxant and stimulant.

- Helps individuals suffering from sleep disorders when combined with Lemmon oil
- Aids in hair care by adding sheen and reducing hair loss
- Helps us skin care,
- To kill fungus, virus and bacteria in our body etc.
- Enhance physical and mental well-being.
- Herbal products in cosmetics or herb in cosmetics can also be referred as botanical origin products in cosmetics(Kooti et al., 2015).

2.6 Criteria of quality of oils and Characterizations

The oil that should have fulfill any of the physical and chemical properties stands for the quality of the oil when we saw the physical properties easily understood with necked eye. The characters of the oil or the composition of the oil indicates quality. When we saw the oil it seems clear, pure colorless(Unal et al., 2013).

The criteria for the seed oil quality are:

• Color - most oils should be clear, colorless and clean. A murky oil is a sign of water being present.

• Odor - often the odors are specific to the areas in which the plant is grown. This makes it very difficult for new producers to enter the market. Relative density, Solubility in ethanol, Content of specific chemicals etc.

The oil properties includes this criterion in some extent the indication of the quality of the oil. Mostly the ingredients of the oil live with its standard for a long time, so as quality must necessaries spatially in cosmetics and pharmacies(Bowes & Zheljazkov, 2005).

2.7 Application of Black pepper Seed oil for Cosmetics

Cosmetics are substances used to enhance the appearance or odor of the human body. Cosmetics include skin-care creams, lotions, powders, perfumes, lipsticks, fingernail and toe nail polish, eye and facial makeup, permanent waves, colored contact lenses, hair colors, hair sprays and gels, deodorants, baby products, bath oils, bubble baths, bath salts, butters and many other types of products(Gediya, Mistry, Patel, Blessy, & Jain, 2011). There are a wide range of herbal cosmetic products to satisfy beauty regime. Adding herbs in cosmetics is very safe for our skin. Herbal cosmetics are in high demand due to the increasing interest of mankind towards them because they are more effective with nil or less side effects, easily available ingredients(Rooit et al., 2010.).

2.8 Cosmetic Composition

Cosmetic compositions (dispersions in an oil or water medium or water-in-oil (W/O) or oil in water (O/W) emulsions) comprise of pigment, essential titanium dioxide and optionally fillers, and fatty binder(Bernardo & Saraiva, 2005). Fatty acids are natural components of skin and are components of a complex that makes up the outermost layer that protects the body against oxidative damage. The body uses omega-6 fatty acids to help treat dry or damaged skin conditions such as eczema. Oleic acid in particular is abundant in skin cells and is a very good medium for transferring nutrients across the skin barrier. Oils rich in oleic acid are generally very absorbent and as a result are in wide use in the skin care industry. Fatty acids are used in cosmetics as emollients, anti-oxidants, and cell regulators. Linolenic acid is considered to promote healthy skin growth and is an anti-inflammatory agent(Wanjari & Waghmare, 2015). Polyphenolic are naturally occurs in the skin of grapes which is believed to be beneficial for health. Flavonoids and tannins, and many of these are used, or have the potential to be used, in skin cosmetics. Cosmetic composition also consists of ingredients such as surfactants, whether non-ionic, cationic, anionic or amphoteric, fragrances or adjuvants such as hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active principles, preservatives, antioxidants, solvents, or coloring materials. The amounts of these various adjuvants are usually from 0.01% to 20% of the total weight of the composition.

2.9 Skin care Cosmetics

Skin oil is to protect the skin against harshness from the environment and any dry conditions of the skin. A skin care oil should aid the skin in carrying out its normal functions, which is, restoring moisture to dry skin, allowing the elimination of waste matter through the pores, and the cooling of the body by evaporation of water (perspiration) and radiation, thus aiding in the maintenance of the normal body temperature(Service & Kong, 2015). Skin disease is common ailments of all age groups because of the infection of a variety of microorganism, chemical agents and biological toxin present in the atmosphere and also due to physical factors, malnutrition and environmental pollution. The raw material from which cosmetics oils are manufactured may either be fresh, partially dehydrated or dried(Hyde & Bahkali, 2010)**.**The skin's pH is naturally acidic, ranging from pH 4 to 6 compared with pH 7.2-7.5 found within the body's internal environment. Lower pH results from the production of certain amino acids and lactic acid by the epidermis, combined

with acidic substances present in sebum and sweat. This acid mantle, as it is known, along with resident microflora, resists chemical and microbial attack by acting as a chemical buffer, detoxifying agent and bacteriostatic(Djisalov, & Oljaca, 2015). In its capacity as a protective barrier the skin also guards the body against damage by ultraviolet light from the sun and other sources, and prevents excessive loss of water from the body which would otherwise lead to dehydration. Such protective properties are greatly reduced or destroyed if the skin is in contact with water, solvents or solutes for long periods of time(Butler, etal., 2009.).

2.10 Characteristics of oil and FTIR Spectra reference zone

Molecular vibrations result in a change in the bond dipole moment Groups like –CH, -C=O, and -CH_3 are readily identified by a single absorption band. Carbonyl group can easily bond with hydrogen and absorption may occur from 1700-1900cm-1.Aromatic hydrocarbons show absorptions in the regions 1600-1585 cm-1 and 1500-1400 cm-1 due to carbon-carbon stretching vibrations in the aromatic ring. Bands in the region 1250-1000 cm-1 are due to C–H in-plane bending, although these bands are too weak to be observed in most aromatic compounds(Hou & Jones, 2000). Alkenes and aromatics show a C–H stretch slightly higher than 3000 cm-1. Aliphatic ketones 1715 cm-1, α, β-unsaturated ketones 1685-1666 cm-1,1750-1735 cm-1 saturated aliphatic esters, 1740-1720 cm-1 saturated aliphatic aldehydes,1730-1715 cm-1 α, β-unsaturated esters.1710-1665 cm-1 α, β-unsaturated aldehydes and ketones.(One, Principle, & Ftir, n.d.). H–C=O stretch 2830-2695 cm-1, Aliphatic aldehydes 1740-1720 cm-1, Alpha, beta-unsaturated aldehydes 1710-1685 cm-1.The O=C–H stretches in both aldehydes in the region 2830-2695 cm-1, the carbonyl stretch C=O of a carboxylic acid appears as an intense band from 1760-1690 cm-1. N–H stretch 3400-3250 cm-1.1° amine: two bands from 3400-3300 and 3330-3250 cm-1. 2° amine: one band from 3350-3310 cm-1. 3° amine: no bands in this region. N–H bend (primary amines only) from 1650-1580 cm-1,C–N stretch (aromatic amines) from 1335-1250 cm-1,C–N stretch (aliphatic amines) from 1250–1020 cm-1.N–H wag (primary and secondary amines only) from 910-665 cm-1.The region from 900-650 cm-1. Aromatics, alkyl halides, carboxylic acids, amines, and amides show moderate or strong absorption bands (bending vibrations) in this region.(Manual, n.d.).

3. MATERIALS AND METHODS

3.1 Materials and Equipments

3.1.1 Materials

The major raw materials used during the experimental works were, Nigrum seed and leaves, filter Paper, Aluminum foil, Na_2So4 NaOH, NaCl, Vaseline,(Mg/KOH), Lemmon oil, gram positive bacterial cells, Ethyl acetate, hexane, alcohol (ethanol and methanol), mineral oil, ethanol,150-mL nutrient agar, the other chemicals used in my experimental work from Addis Ababa university.

3.1.2 Equipments

For my experimental work I used the equipments, Storage and separator tank, distillation unit, cutter, beaker (3),250-mL beaker (3),beaker tongs, volumetric flask, decanter, weight machine, GC_MS, FTIR, condenser, measuring cylinders, burette, stirring rod, petri dish, thermometer, 110°C, the other equipments were used from Addis Ababa university laboratories.

3.2 Raw Material Collection and Preparation

3.2.1 Raw Material Collection

The raw of Black pepper (nigrum) seeds collected from the shewarobit far from 90 km from debrebrhan and debremarkos far from bahrdar. The lab work were also performed Addis Ababa University and Debrebrhan University. Due to the reason of acessesability and potential of the crop were highly planted.

Figures 3.1: collected raw materials

In shewarobit, I didn't get the materials but I get the leave from the farmer's market garden.it also highly planted in shewarobit in farmer's garden. I used (10 kg) of raw material with characteristic, and has a distinctive odor.

3.2.2 Raw Material Preparations

After the collection of the seed and the leave it was stays for two days for moisture and aroma odor removing and keeping the color as green. The condition of setting free to the environment and also a little bet the aroma odor of leave be decrease. The seed would be dried in drier or oven at temperature of 105^{o}c by using the given temperature. Before drying the nigrum leave and seed the color would be green.

Figures 3.2: crushed raw materials with different size

The materials were crushed by using milling machine in the lab. The crushed materials are screen out by using sieve. Different sieve would be selected in my lab work to determine the size of the particle for the next step. The size of the solid material was small enough, thus, my selected sieve analysis for my lab were different ,but after screening the average size of the particle for my work 500μm, 750μm and 1mm particle size was selected. According to the average size of the particle sex sieves were selected for sieve analysis. Three particle size samples were screened.

3.3 proximate Analysis of Nigrum Seeds and Leaves

A. moisture content

The seeds and leave moisture content were done by using oven at the temperature of 105^{o}c, for one days for better removal of moistures. The weight of the raw materials were done by using the following equations.

Moisture content% = (the amount of samples before drying-the amount of samples after drying/the amount of samples)*100

B. **Ash content**

The crushed nigrum seed weight were known before inserted into the furnace to determine the ash content

The muffle furnace was heated up to a temperature of 650℃. Crucible was preheated about ten minutes, the sample was heated at 650°C in an open crucible for 1 hour 30 minutes in a furnace. The weight of the sample before heating and after heating was used to determine the amount of ash content present in the sample.

$$\text{Ash content\%} = \frac{A}{B} * 100 \quad \text{……..eqn (3.1)}$$

Where A, stands for residue or after drying weight of crushed seed. B, weight of sample before drying.

3.4 Extraction of Essential Oil

The Nigrum trees were used for money purpose from different parts of trees. Its stems, leaves, and seeds as the form of oil and juice. The black pepper oil were money applications such as condiment, traditional medicine, for drying injured body, for cosmetics etc. Based on the literature review the Nigrum (stems, leaves, and seeds) had so money merits. The ingredients of Nigrum oil from its parts of the body had been similar but the percentage compositions are different. The extraction process were done with detail experiment work.

1000gm of seed was measured from each particle size.

The fine particles 500μm, was prepared about 500gm.this samples were divide into two equal amount about 250gm.the samples were introduced to distillation with its proportion. Then the distillation process was started. Before started the distillation the temperature of distiller around 105^{o}c. After 35 minute the first bubble were started to drop into decanter at this temperature. The point of extraction for this size was minimum. After heating for 3hr and 30 minute at the temperature of 105^{o}c highly vaporized gas was flow into condenser unit at the first run, this indicate the maximum temperature and time of this size by using ethanol and water mixture solvent Based on the ratio solid to liquid ratio. The advantages of the mixture solvent was to increase the extraction efficiency by reducing extraction time.

For the same procedure by changed the particle size and temperature with also change distillation time the high yield amount was extracted. For temperature of 127.5^{o}c and the time from3:30, 4:00, 4:30hr with particle size 750micro.m, 1mm the extraction was performed with the same procedures. For the temperature of 150^{o}c for this particle size with differentiate time the extraction was done. For the other particle size the similar procedures to perform optimum yield and optimum amount of oil in ml. the ratio of solid to liquid was 1:3 and the solvent of ethanol to distilled water was 1:2.

Yield% = (mass of oil after extraction/raw samples introduced into distillation)*100 ……eqn (3.2)

3.4.1 Optimization of the Extraction Process

The extracted oil were done with the temperature of 127.5^{o}c the particle size of 750micro.m and time also 4:30hr to extracted high yield of oil. When the time factor was increased the maximum amount of yield was recorded. The same as temperature of the steam distillation was at 127.5^{o}c with this time maximum yield or amount of oil in ml was extracted at this particle size.

3.4.2 Sample Analysis and Modeling Equations

A. sample analysis

The raw material sample were analysis by using FITR, and gas chromatography and Mass Spectrometer apparatus for the sample of oils analysis, which, evaluates cosmetics Oil qualitatively and quantitatively. The density and specific density were measure by using hydrometer. Particle size distribution of sample made by using sieve analysis. Crushed raw materials were changed in the powder form with different size. The mass of the sample was measured in grams, 1000gm, samples were measured from different size of particles. Then next procedure was applied. The samples were get ready for the extraction, the measured samples with its size were 500µm, 750µm, 1 mm, 500gm,500gm and 500gm respectively. Each samples were replicate into two then measured about 250gm from the samples and each size. 250ml of ethanol (90%) and 500ml distilled water for 250gm of samples from each particle size. The purpose of the solvent to increase the efficiency of the extraction.

B. model equations

For my work there was some model equations to analysis the result of the extraction condition.

Total sum of square

$$SST = \sum_{k=1}^{n}(Xi - X)2 \ldots\ldots\ldots\ldots\ldots\ldots\text{eqn (3.3)}$$

SST = SS $_{Treatments}$ + SSE, sum of squares due to treatments+ *sum of squares due to errors.*

$$SSE = \sum_{k=1}^{n}(Xi - X)2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{eqn (3.4)}$$

Where X= sample variables.

$ms\ treatment = \frac{sstreatment}{a-1}$……eqn (3.5), a-1 means degree of freedom, a=no of sample freedom. MS=mean squares.

A rough check for outliers made by standardized residuals**.**

$Dij = \frac{eij}{MSE} = MSE = \frac{SSE}{N-a}$……..eqn (3.6) N=no of samples. Eij =error of percent or confidence interval.

,

3.5 Preparation of Cosmetics Oil

The essential oil of black pepper leave was similar with seed oil based on the experiment result but in some extent it was different according to the composition such as composition, retention time during the analysis of GC_MS, fatty acid content etc. for preparation of cosmetics both oil were advantageous. But in my experiment I selected seed oil. The mixer was the important machine to mix the oil, the Lemmon oil and distilled water were mixed at 75^{o}c.the density of Lemmon oil 0.8g/ml. total amount of extracted oil from Lemmon was 60ml from 250gm and total extracted oil from nigrum seed and leave were 200ml and 170ml respectively. The blending mechanism was not much difficult, first the amount of seed oil separately known.2ml of seed oil were prepared. The Lemmon oil and distilled water were changed into liquid before mixing the oil by using steam distiller, this tends to for knowing of the amount of solution ration in ml then 22ml of solvent were prepared. After knowing the amount of each samples the Lemmon oil to distill water ration 1:5 due to the reason of pH value at this ratio the pH becomes 6.1.the amount of Lemmon oil 4ml and 18ml of distilled water were mixed. The prepared the seed oil and the solution were mixed by checking the pH value with mixer after the ration 1:7 the PH become 5.3. The purpose of addition Lemmon oil into the Nigrum oil were to change the aroma into good odor and to be concentrated the oil due to the reason of low yield. But firstly checked the pH ration before mixed into the oil, after adjusted of the pH of solvent.

3.6 cosmetics oil Characterization

The Nigrum oils were characterized by the FTIR, GC_MS, and acid value. The physio chemical properties of the oil were analysis by GC-MS and acid value NaOH were an important index of physicochemical properties, and the anti- bacterial properties were also evaluated.

A. Characterizations of the physico-chemical properties

The color of the oil that was clear, colorless indicates the quality of the oil. And also the method of extraction were selectable. The FTIR spectra were stretching unsaturated fatty acids, aromatics, amines. From the GC_MS result oleic acid, 9, 12-octadecadienoic acid [Z, Z], Octadecanoic acid, 9-octadecenoic acid,(E)-cis-13-octadecenoic,n—Hexadecanoicacid, 4,4,8 trimethyltricyclo [6.3.1.0(1,5) dodecane-2,9-diol,5-pyrrolidin-2-ylidenemethyl-3,4-dihydropyrrol 2-one,ledene

oxide-(I), (z)-2,6,6-trimethyl-.alpha.-(1-propenyl)-2-cyclohexane-1-methanol,4-(1,3-dimethyl-3-cyclohexanyl)-1,6heptadien-4-ol, were highly covered.

B. Anti- bacterial properties of oil

Microorganisms were obtained from the Department of Microbiology from Ethiopian biodiversity institute. The staphylococcus aureus bacteria had been gram positive spices and cocci in shape. It was cultured at 37^{o}c and the nutrient agar had best for culturing this bacteria for 24hr with petri dish by made serial dilution. Before doing every work each necessarily materials had been sterilize, 11 petri-dish and nutrient agar sterilize for 121^{o}c for 15minute. The sterilization purpose would be to killed unwanted bacteria and other living things. The anti-bacterial properties of the oil characterized against by added the oil into the cultured bacteria. After that amount of oil addition 0.25-3ml of oil by dropped into the petri dish then observe the condition.

The amount of bacterial cell from this petri dish:

Number of bacteria=number of colonies/dilution factor

C. Acid value

After done all things I checked the acidity of the essential fennel oil.

Take 10 gm or 16ml of Nigrum oil mixed in accurately weighed by using 60^{o}c temp, in 16 ml mixture of equal volume of alcohol but the alcohol concentration 97% and solvent hexane, the flask was connected to reflux condenser and slowly heated, until sample was dissolved completely, to this three drop of phenolphthalein added and titrated with 0.1N NaOH, until the color changed pink color appears after shaking for 30 seconds. I performed three run to select the best result.

Acid value = V*N*5.61/W…….eqn (3.7).

Where V volume of expressed in milliliter of 0.1N solution of ethanoic NaOH.

N, concentration of the NaOH. W, weight of the oil

3.7 Experimental Design and Statistical Analysis

During the extraction of the black pepper seed oil, three different distillation temperatures between 105^{o}C, 127.5^{o}c and 150^{o}C were used and the experiment were used center point. The experimental design on the product characterization was a full factorial design. That means, three factors (distillation temperatures, particle size and extraction time) and two levels (selected high and low values) were used.

Experimental data analysis was done using Design- Expert 7.0.0 software. The experimental design selected for this study was response surface methodology, three-level-three-factor full factorial design and the response variable measured was the percentage of conversion. In addition analysis of physicochemical properties of oil such as FTIR spectroscopy, GC_MS was done at fecality of natural science department of chemistry, Addis Ababa University and Leather Industry Development Institute respectively.

Code terms of design expert				
	Code terms	Particle size	temperature [^{o}c]	time[hr.]
Low code	-1	500micro.m	105	3:30
Center code	0	750micro.m	127.5	4:00
High code	1	1000micro.m	150	4:30

Table 3.1: experiment design matrix

Std	run no	Type	factor 1 A.p.size,mm	factor2 B.temp.^{o}c	factor3, C.time(hr)
1	1	Fact	-1.00	-1.00	-1.00
2	25	CentEdge	0.00	-1.00	-1.00
3	29	Fact	1.00	-1.00	-1.00
4	14	CentEdge	-1.00	0.00	-1.00
5	17	CentEdge	0.00	0.00	-1.00
6	27	CentEdge	1.00	0.00	-1.00
7	26	Fact	-1.00	1.00	-1.00
8	8	CentEdge	0.00	1.00	-1.00
9	13	Fact	1.00	1.00	-1.00
10	12	CentEdge	-1.00	-1.00	0.00
11	16	CentEdge	0.00	-1.00	0.00

12	30	CentEdge	1.00	-1.00	0.00
13	20	CentEdge	-1.00	0.00	0.00
14	6	Center	0.00	0.00	0.00
15	4	CentEdge	1.00	0.00	0.00
16	7	CentEdge	-1.00	1.00	0.00
17	9	CentEdge	0.00	1.00	0.00
18	2	CentEdge	1.00	1.00	0.00
19	19	Fact	-1.00	-1.00	1.00
20	32	CentEdge	0.00	-1.00	1.00
21	18	Fact	1.00	-1.00	1.00
22	28	CentEdge	-1.00	0.00	1.00
23	22	CentEdge	0.00	0.00	1.00
24	31	CentEdge	1.00	0.00	1.00
25	3	Fact	-1.00	1.00	1.00
26	24	CentEdge	0.00	1.00	1.00
27	11	Fact	1.00	1.00	1.00
28	5	Center	0.00	0.00	0.00
29	15	Center	0.00	0.00	0.00
30	10	Center	0.00	0.00	0.00
31	23	Center	0.00	0.00	0.00
32	21	Center	0.00	0.00	0.00

3.7.1 Experimental set up

In this experiment work the steam distillation extraction process were used at the temperature of 105,127.5 and 150^{o}c, at the time of three level which 3:30, 4:00, and 4:30hr selection. For best extracted oil the particle size 500,750,1000micro.m for my selection based on literature review.

Figure: 3.3 experimental set up

3.7.2 Statistical Analysis

The statistical work were done after the experimental design work for the response surface model. In my work the yield was analysis by ANOVA for the knowing of the variance, to knows the interaction of the factors, coherence, percent contribution, the R-squared, LSD, degree of freedom, all about this things were done with ANOVA statistical method.

Test statistics

$$fo = \frac{SStreatment(a-1)}{SSE(N-a)} = \frac{MStreatment}{MSE} \quad \text{eqn (3.8)}$$

The analysis of variance summary at the computer output contains the usual sums of squares, degrees of freedom, mean squares, and test statistic *FO*. The column' 'Prob > F" is the P-value (actually, the upper bound on the P-value, because probabilities less than 0.0001 are defaulted to 0.0001).In addition to the basic analysis of variance, the program displays some additional useful information. The quantity "R-squared"

$$R^2 = \frac{SSmodel}{SStotal} \quad \text{eqn (3.9)}$$

R-squared indicates the closeness of the factors for the model, it approach to 1means good fits for the model selection.

4. RESULT AND DISCUSSION

4.1 Proximate Analysis of the black pepper (nigrum) Seeds and Leaves

At the temperature of 105^{o}c the seed of nigrum were dried. At this temperature of nigrum was dried. Before introduce into the oven the mass of both the seed and the leave were measured. After drying the seed color become gray and the leave color also gray the mass of both samples were measured. There was some calculation about the samples to know moisture content. The amount of seed were about 4kg and the leave was about 3.5 kg for experimental work. After dried the samples at 105^{o}c the seed amount become 3.86kg, and the leave amount also 3.246kg but the samples were dried 24hours for both samples. The dried time would selected randomly to get good samples because the drying time would increase the samples were highly dried and quality of samples.

The moisture content as follows:

((4kg-3.86kg)/4kg)*100%=3.5%, the moisture content of leave was as follows: ((3.5kg-3.246kg)/3.5kg)*100%=7.257%. It would be prepared the samples for extracted oil. The seed were seasonally from November up to December. Otherwise the raw seeds not fully cropped.

Ash content of both seed and leave from 100gm for each samples were 3 and 2.2% respectively. This indicates that the inorganic substance were not affect the extraction process and also there had not the effect on the yield or the amount of oil in ml.

4.2 Extraction process of Black Pepper Oil

Firstly the 500µm size run into the distillation unit by adjusting the temp. 105^{o}c for 3:30hr the amount of oil became 6.63ml so as run with this temp. After the next run a little minimum by waiting for four hour the amount became increase up to 9.05ml of oil extracted. The next day extracted of the oil was for 4hr and 30 minute and the temperature of 105^{o}c to become down or extracted process become stopped. The separation of oil from ethanol and water solvent was performed by density difference for all extraction process.

The maximum amount of oil for this particle for the first run by using 105^{o}c the result was 10.72ml from 250gm samples until the time run up to 4:30hr.

The next samples for this 500μm size, 250gm was introduced into the distillation unit, there was some temperature adjustment about 127.5^{o}C. After started distillation process at 25minute the bubble was stand to condensed into the decanter to drop as distillate, then after 3hr and 30 minute the maximum amount of distillate was dropped into decanter at this the amount become after 3hr 30minute the distillation process was stopped but the amount of oil was 12.74ml.after the following day the same particle size and the same amount to be introduced into distillation unit at the temperature 127.5^{o}c.the first distillate was started to dropped into decanter at 27minute.the maximum extraction was performed at 4hrs and 30minute.distillation process was stopped at 4hrs and 30minute.the amount of oil after extraction was calculated below in ml.

YIELD(Y) % = (1-AS/AO)*100 ……..eqn (4.1)

AO=the amount of raw materials in mass to introduce in the distillation by % and AS= amount of substance after extraction the mass raw materials in %. Or in other mechanism.

Y= (Mass of extracted oil in mass/ mass of raw materials introduced in distillation)*100%.

Total Yield = (1-total amount of raw materials after extraction/amount of samples introduced into distillation)*100% but the best determination case was the second method because easily determine the highest of nigrum seed oil content:

We calculated nigrum oil yield separately for 500μm particle size, (amount of oil in mass/amount feed)*100%=

For 1st run,at105^{o}c, mass of 10.72ml for4:30hr distillation time=6.75gm, yield=(6.75gm/250gm)*100=2.7%.for 4:00hr the same particle size and temperature's the amount become 9.05ml the mass also 5.7gm yield become=(5.7/250)*100=2.28%

At 3:30hr 6.63ml of oil in mass 4.175gm yield become=1.67%.

The total amount of oil from the experiment, at this particle size and 105^{o}c three time interval: (6.63+9.05+10.72) ml=26.4ml or 16.63gm.

Yield from total amount of oil and sample= (16.63/750)*100=2.2%.

127.5^{o}c, at the time of 3:30hr the amount of oil 12.74ml oil=8.02597gm,

Yield = (8.026/250gm)*100=3.21%. At the same temp. But in different hr. until 4:00hr duration 12.90ml of oil extracted the mass would be 8.125gm the yield= (8.125/250)*100 =3.25, up to the

of 4:30hr 13.90ml of oil extracted. Yield become=3.502%.the total amount of oil from this temperature's=39.53ml.150^{o}c, mass of 12.58ml up to time duration 3:30hr=7.53gm, content= (7.925/250gm)*100=3.17%,the 2nd run also performed at this temperature's and same size and duration the amount become=13.1ml of oil extracted in mass 8.25gm the yield also 3.3%, after 4:00hr. The amount of oil become12.90ml, and in mass 8.125gm in the first experiment. The yield become=3.25, when the time raise into 4:30hr the amount of oil would 13.8ml and in mass 8.7gm the yield also 3.48%. For this particle size the optimum temperature was 127.5^{o}c, and time 4:30hr based on the amount of yield.

The next particle size 750µm would be measured about 1kg sample of powder and 250gm would be introduced into distiller with solvent proportion. Then after heated up until the first droplet of vapor started to condense. At the temperature of 105^{o}c and 53 minute started to extract the droplet oil. It was good condition process. The temperature would constant until stopped the extraction process about 105^{o}c at the time of 3 and 30 minute extraction process to become good enough or high amount of vapor stands too vaporized into the condenser. This indicates that the distillation process was maximum or saturation point was known. After several minute the process of extraction to became slow down. At the time of 4hr and 30minute the extraction oil or the volatile materials were stopped. Finally the oil was separated by density difference. The amount of oil had been from each duration were listed below.11.5ml.This tends too led for oil extraction at high temperature and enough time no more extraction efficiency since to comparison of 500µm.

Then after at 127.5^{o}c the distillation process repeated using the same amount of samples the maximum time 3hr 30minute to stop extraction. The amount of oil was 14.75ml. When we did the same amount and size but different temperature at 150^{o}c.the consumption time to conclude the extraction was 3hr and 35minute but the amount of oil was 11.75ml.

Total amount of oil =11.75+14.75+11.5=38ml, to get the yield calculation changed the measured oil in grams.38ml oil 24gm.

Total amount of content from this size = (24gm/750)*100=3.2%.

For each temperature the amount of yield:-

Table 4.1: summary of experiment result from steam distillation

Size		Temperature	Amount in ml	Amount in gm	Time	Yield%
750	250gm for each run	105	9.52	6	3:30	2.407
			11.5	7.244	4:00	2.897
				7.975	4:30	3.19

a) 105^{o}c, 11.5ml=7.244gm, yield =(7.244gm/250)*100%=2.897%
b) 127.5^{o}c,14.75ml=9.29gm, yield=(9.29/250)*100%=3.716%
c) 150^{o}c,11.75ml=7.4gm, yield=(7.4/250)*100%=2.96%

From this size the high amount of yield was from 127.5^{o}c temperature.

For 1mm particle size the distillation process as follows.

1000gm of powder samples would be measured. 250gm introduced to the distillation unit at 105^{o}c the first distillate was recorded at one hr. After 4hr and 30minute the maximum extraction would be finished. Finally the extraction of this was slow down after 4hr 30minute the temperature still 105^{o}c. in this particle size the time and the temperature were highly disordered. The amount of oil 8ml. for the second temperature at 127.5^{o}c still the same amount of sample and size was selected but 10.5ml oil was extracted at the time of 4hr. the last temperature range was 150^{o}c was adjusted by introducing the same amount of sample in this type of size. The amount of oil from this temperature was 9.2ml. This size would be gave the amount oil below all type of size.

The total oil from this size was (9.2+8+10.5) ml=27.7ml. In general for all size the separation of oil from some nonvolatile and water was done by density separation method using decanter. The optimum oil was extracted from the size of 750μm based on the quantity and yield. The temperature selection should be between 100-200^{o}C from essential oil standard temperature. The size also from 0.5mm up to 2.5mm from the standard of the oil particle size to get the best size for essential oil. The extraction of oil from leave was performed by same analysis. The optimum temperature and time was the same. But the best size was selected based on seed size performance.

The size of the leave would be 750μm and introduced into the distillation unit after 26minute and 127.5^{o}c the first extraction was started. It was different in some extent from seed in extraction process and parameters the optimum condition of extraction was about 3hrand 45minute. For leave some difference was existed based on yield content. For leave at 127.5^{o}c and 750μm size distribution the following things were a little difference. But the density of leave oil and seed oil almost the same 643kg/m^3 and 640kg/m^3 respectively. So almost equal. The density would .643gm/ml. the pH of the oil 4.7, the PH of the Lemmon oil almost the same with the PH of human body it is not acidic and also so it nearest to the skin care cosmetics specifications.

4.3 Statistical Analysis of factors Extraction Black Pepper Seed oil

Table 4.2: design expert analysis of the yield

Code terms of design expert				
	Code terms	Particle size	temperature [^{o}c]	time[hr.]
Low code	-1	500micro.m	105	3:30
Center code	0	750micro.m	127.5	4:00
High code	1	1000micro.m	150	4:30

Std	run no	type	factor 1 A.p.size,mm	factor2 B.temp.^{o}c	factor3, C.time(hr)	Yield(%)
1	1	Fact	-1.00	-1.00	-1.00	1.67
2	25	CentEdge	0.00	-1.00	-1.00	2.407
3	29	Fact	1.00	-1.00	-1.00	1.8
4	14	CentEdge	-1.00	0.00	-1.00	3.21
5	17	CentEdge	0.00	0.00	-1.00	3.716
6	27	CentEdge	1.00	0.00	-1.00	2.15
7	26	Fact	-1.00	1.00	-1.00	3.17
8	8	CentEdge	0.00	1.00	-1.00	3.7
9	13	Fact	1.00	1.00	-1.00	2.5
10	12	CentEdge	-1.00	-1.00	0.00	2.28
11	16	CentEdge	0.00	-1.00	0.00	2.8

12	30	CentEdge	1.00	-1.00	0.00	2.27
13	20	CentEdge	-1.00	0.00	0.00	3.25
14	6	Center	0.00	0.00	0.00	4.7
15	4	CentEdge	1.00	0.00	0.00	2.33
16	7	CentEdge	-1.00	1.00	0.00	3.36
17	9	CentEdge	0.00	1.00	0.00	4.09
18	2	CentEdge	1.00	1.00	0.00	3.07
19	19	Fact	-1.00	-1.00	1.00	2.7
20	32	CentEdge	0.00	-1.00	1.00	3.35
21	18	Fact	1.00	-1.00	1.00	2.45
22	28	CentEdge	-1.00	0.00	1.00	3.3
23	22	CentEdge	0.00	0.00	1.00	4.83
24	31	CentEdge	1.00	0.00	1.00	2.6
25	3	Fact	-1.00	1.00	1.00	3.48
26	24	CentEdge	0.00	1.00	1.00	4.15
27	11	Fact	1.00	1.00	1.00	3.15
28	5	Center	0.00	0.00	0.00	4.67
29	15	Center	0.00	0.00	0.00	4.3
30	10	Center	0.00	0.00	0.00	4.45
31	23	Center	0.00	0.00	0.00	4.27
32	21	Center	0.00	0.00	0.00	4.56

DesignSummary

Study Type Response Surface Experiments 32
Initial Design 3 Level Factorial Blocks No Blocks
Center Points 6
Design Model 3FI

Response	Name	Units	Obs	Minimum	Maximum	Trans	Model
Y1	yield	%	32	1.67	4.83	None	No model chosen

Factor Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded
A p.size	mm	Numeric	-1.00	1.00	-1.000	1.000
B.temprature	oc	Numeric	-1.00	1.00	-1.000	1.000
C.time	hr	Numeric	-1.00	1.00	-1.000	1.000

Response: yield
Sequential Model Sum of Squares

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Mean	342.78	1	342.78			
Linear	7.17	3	2.39	3.68	0.0236	
2FI	0.24	3	0.081	0.11	0.9517	
Quadratic	15.66	3	5.22	50.29	< 0.0001	Suggested
Cubic	0.66	7	0.094	0.87	0.5518	Aliased
Residual	1.62	15	0.11			
Total	368.14	32	11.50			

"Sequential Model Sum of Squares": Select the highest order polynomial where the additional terms were significant and the model was not aliased.

Lack of Fit Tests

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Linear	18.02	23	0.78	23.39	0.0012	
2FI	17.77	20	0.89	26.53	0.0009	
Quadratic	2.12	17	0.12	3.72	0.0763	Suggested
Cubic	1.46	10	0.15	4.35	0.0592	Aliased
Pure Error	0.17	5	0.033			

"Lack of Fit Tests": Want the selected model to had insignificant lack-of-fit.

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.81	0.2829	0.2061	0.1191	22.34	
2FI	0.85	0.2925	0.1227	-0.1425	28.97	
Quadratic	0.32	0.9100	0.8731	0.8117	4.77	Suggested
Cubic	0.33	0.9359	0.8676	0.6258	9.49	Aliased

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".The predicted R-squared indictes the the closeness of the factors for the model, it approach to 1 means good fit for the model selection, quadratic model was fit for our work. The standard devation indictes the difference between each factors from temp,particle size,time and grouped factor difference.so from the difference we take the smallest value in number.

From the above smallest in number were quadratic model so we selected it.

Tables 4.3: The effect of the factors

model	Term	DF	Sum of squares	Mean square	F value	Prob>f	%contribution
M	A	2	14.07	7.04	210.08	<0.0001	55.5
M	B	2	6.7	3.35	99.99	<0.0001	26.42
M	C	2	2.06	1.03	30.74	0.0016	8.12
M	AB	4	1.80	0.45	13.42	0.0070	7.09
E	AC	4	0.11	0.028	0.84	0.5534	0.45
E	BC	4	0.15	0.037	1.09	0.4508	0.58
E	Pure error	5	0.17	0.033			0.66
	Residuals	5	0.17	0.033			

The ‘m’ indicates the model and ‘e’ value’s errors the effect analysis to know the %contribution in the experiment. From the above model the main factors should be highly contribute in the process, specifically, temperatures and particle size were highly affected the extraction process in this steam distillation. But when we saw the interaction of each factors there was small contribution in the process except the temperature and particle size from the above tables. After the model selection the ANOVA analysis below.

ANOVA statistics analysis of the model:

Response: yield
ANOVA for Response Surface Quadratic Model
Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF 9	Mean Square	F Value	Prob > F < 0.0001	significant
Model	23.07		2.56	24.71		
A	0.93	1	0.93	9.00	0.0066	
B	4.44	1	4.44	42.82	<0.0001	
C	1.80	1	1.80	17.31	0.0004	
A^2	9.03	1	9.03	87.05	<0.0001	
B^2	1.89	1	1.89	18.23	0.0003	
C^2	0.26	1	0.26	2.53	0.1261	
AB	0.11	1	0.11	1.08	0.3099	
AC	8.533E-003	1	8.533E-003	0.082	0.7770	
BC	0.12	1	0.12	1.18	0.2888	
Residual	2.28	22	0.10			
Lack of Fit	2.12	17	0.12	3.72	0.0763	not significant
Pure Error	0.17	5	0.033			
Cor Total	25.36	31				

The Model F-value of 24.71 implies the model was significant. There was 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 were significant. In this case A, B, C, A^2, B^2 were significant model terms. Values greater than 0.1000 indicate the model terms were not significant. If there were many insignificant model terms (not counting those required to support hierarchy),model reduction may improve your model. From the above coded terms ,temprature,particle size,time and the square sum of the both particle size and tempratures were the main factors that were significantly affect the extraction.

The "Lack of Fit F-value" of 3.72 implies there is a 7.63% chance that a "Lack of Fit.

F-value" this large could occur due to noise. Lack of fit is bad -- we want the model to fit.

Std. Dev.	0.32	R-Squared	0.9100
Mean	3.27	Adj R-Squared	0.8731
C.V.	9.84	Pred R-Squared	0.8117
PRESS	4.77	Adeq Precision	16.801

The "Pred R-Squared" of 0.8117 is in reasonable agreement with the "Adj R-Squared" of 0.8731.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 was desirable. Your ratio of 16.801 indicates an adequate signal. This model could be used to navigate the design space.

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.30	1	0.11	4.08	4.53	
A-p.size	-0.23	1	0.076	-0.39	-0.070	1.00
B-temprature	0.50	1	0.076	0.34	0.65	1.00
C-time	0.32	1	0.076	0.16	0.47	1.00
A^2	-1.12	1	0.12	-1.37	-0.87	1.10
B^2	-0.51	1	0.12	-0.76	-0.26	1.10
C^2	-0.19	1	0.12	-0.44	0.058	1.10
AB	-0.097	1	0.093	-0.29	0.096	1.00
AC	0.027	1	0.093	-0.17	0.22	1.00
BC	-0.10	1	0.093	-0.29	0.092	1.00

4.3.1 Check of Model Adquacy and Model Equations

Final Equation in Terms of Coded Factors:

yield =+4.30-0.2* A+0.5* B+0.3* C-1.12 * A^2-0.51* B^2-0.19* C^2-0.097 * A * B+0.027 * A * C -0.10* B *C.

Final Equation in Terms of Actual Factors:

yield =+4.30195-0.22778 * p.size+0.49683* temprature+0.31594* time-1.12369* $p.size^2$ -0.51419*$temprature^2$-0.19153* $time^2$-0.096667* p.size * temprature+0.026667* p.size * time -0.10108*temprature * time.

The predicted R-squared indictes the the closeness of the factors for the model, R-Squared0.9100,Adj R-Squared ,0.8731,Pred R-Squared.0.8117it approach to 1 means good fit for the model selection, quadratic model was fit for our work.The standard devation indictes the difference between each factors from temp,particle size,time and grouped factor difference.so from the difference I took the smallest value in number.From the above smallest in number are quadratic model so we selected it. The interaction graph below shows the temperatures and particle size with time affected the yield of the oil, from the graph the factors B,A and the interaction of AB,with time involvment were highly contribution.

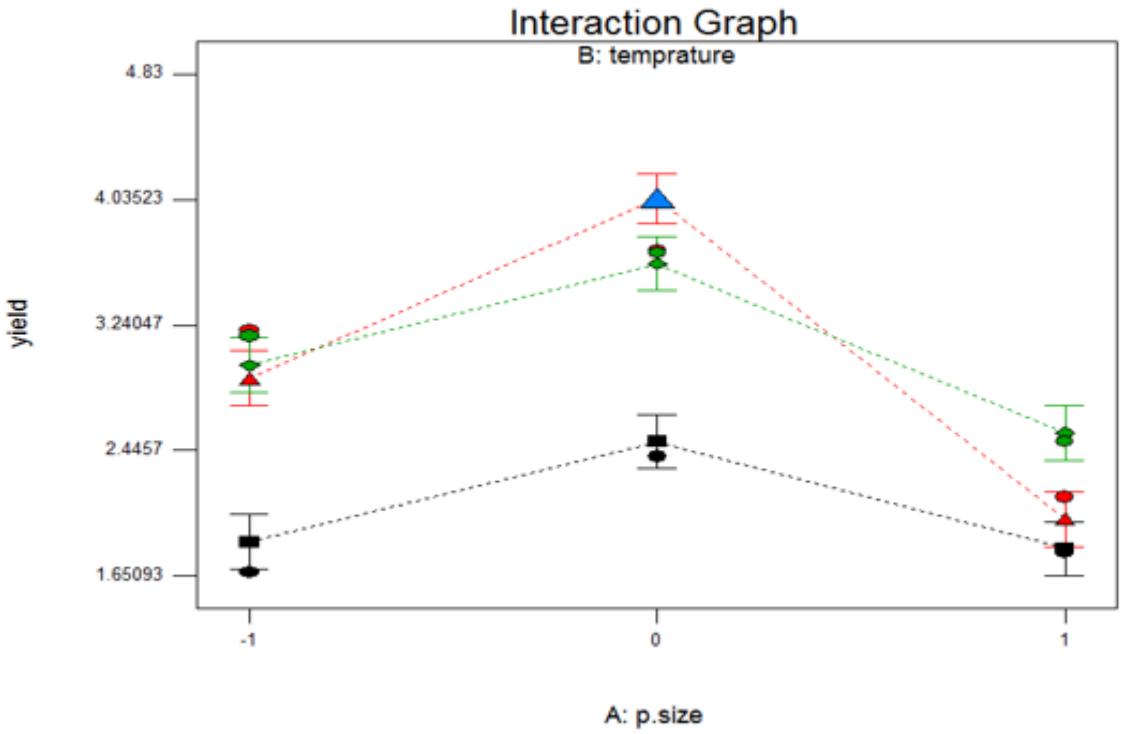


Figure 4.1: Interaction of temperatures and particle size vs yield

From the above interaction graph the maximum yield would 4.04, at the temperature of 127.5^{o}c.the three desgin points the green, red and black color indicates the temperatures black color the 105,green150 and red color 127.5, at the particle size 750micro.m,the value of LSD 0.138.LSD indicates the mean of the sample data should greater than the indicated value on the graph.

So the LSD value from the interaction graph indicates this condition. The coded factors -1, 0, 1 indicates also particles size of 500micro.m, 750 micro. M, 1mm respectively.

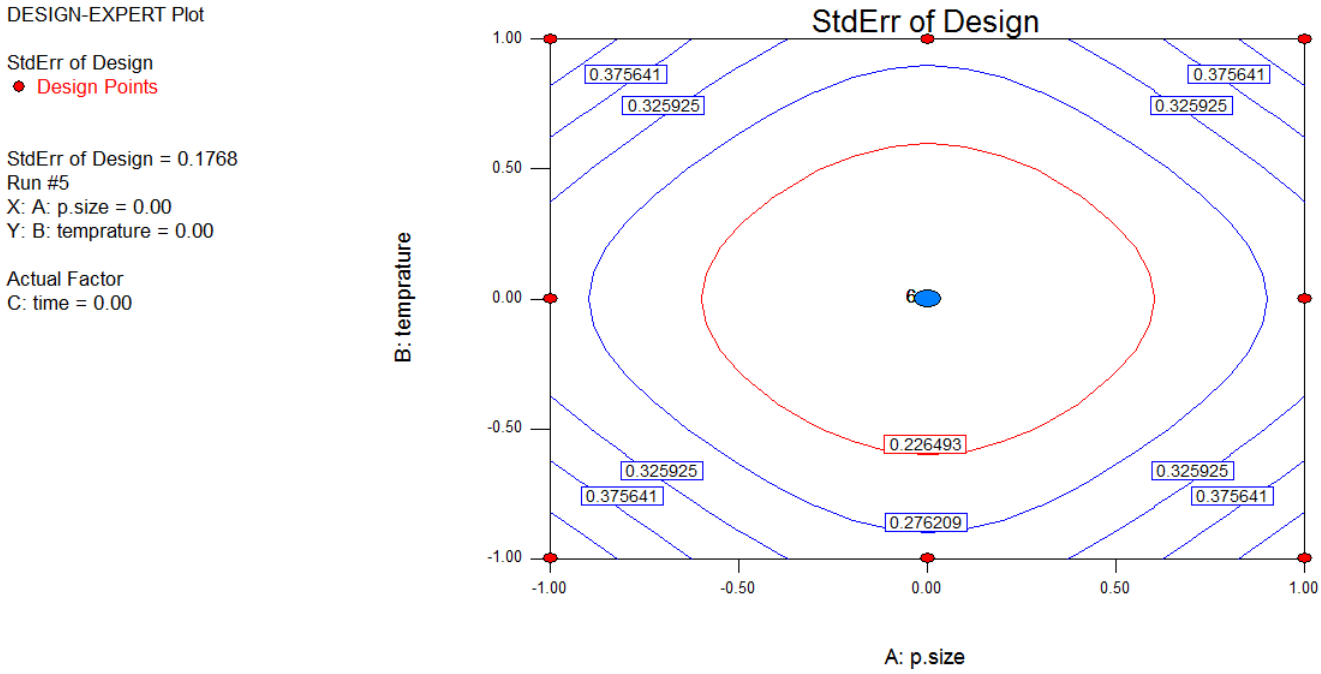


Figure 4.2: the counter graph of standard design error with the p. size vs temperature.

At the optimum point,the p.size,temperature,and time ,0[750],0[127.5],0[4:00] respectively, the standared error desgin 0.1768 besed on the graph result.the red circle in the counter graph maximum yield result performed with small standared error.

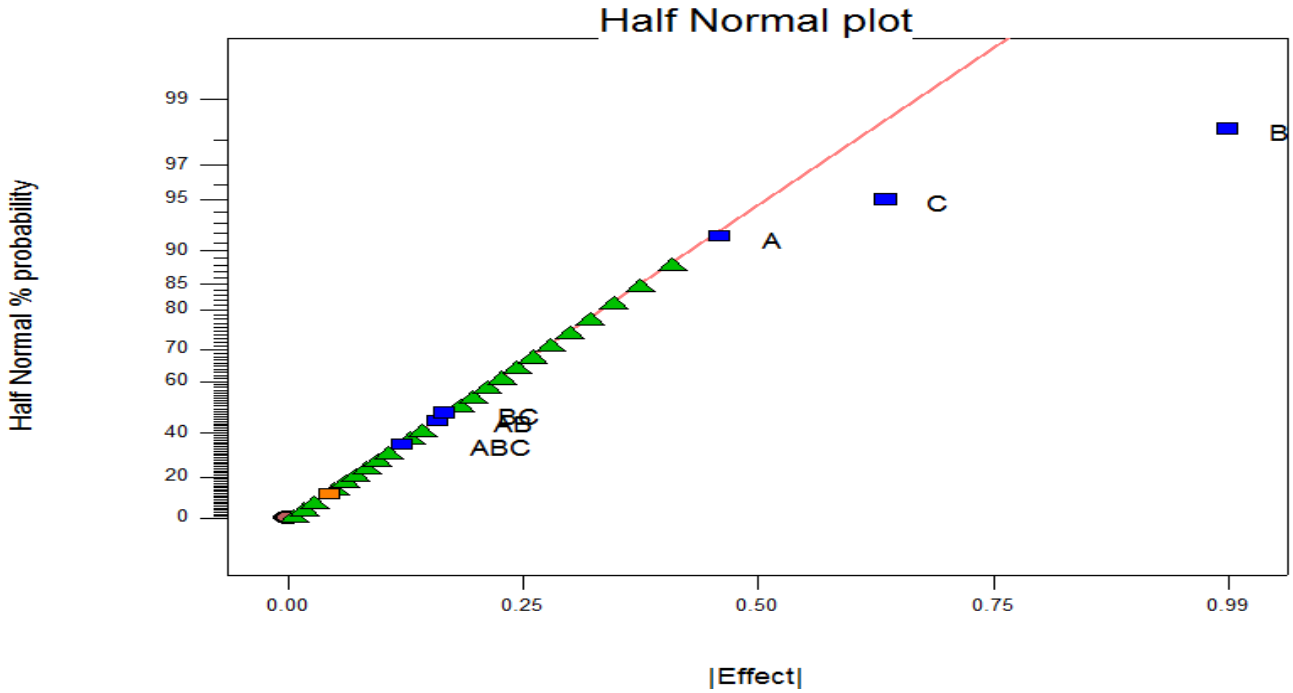
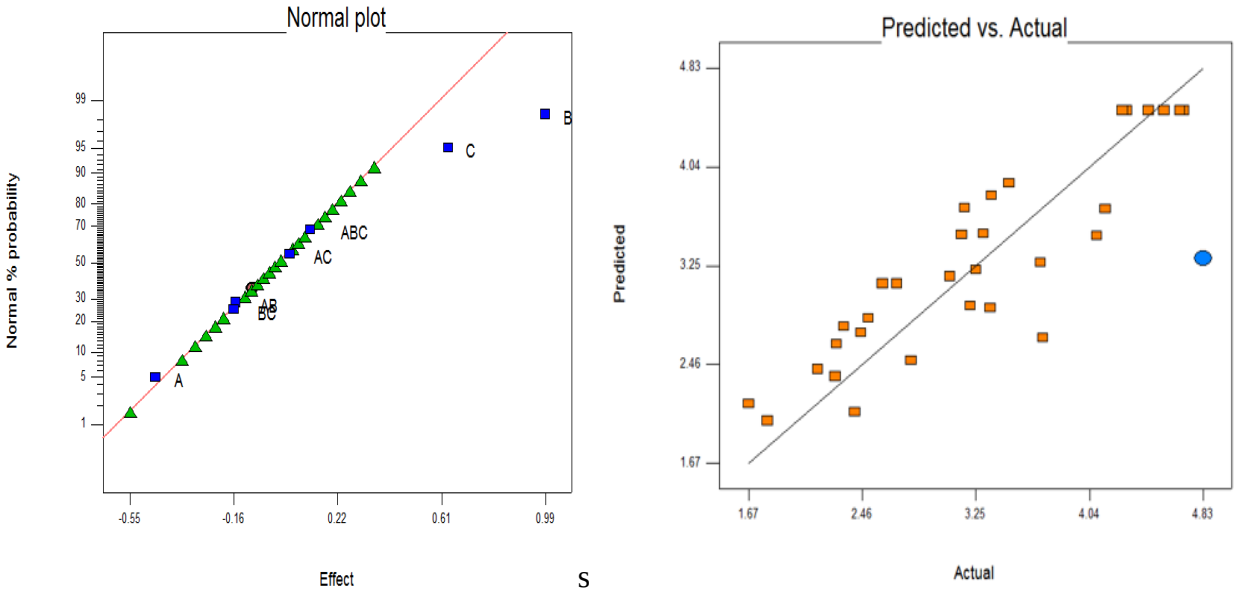


Figure 4.3: half normal probability vs effect interaction graph.

The half normal %probablity versus effect graph shows what were the first factors to affect highly the extraction process,B,C,A,BC,AD, from this code,temperatures were highly affected the yield of the oil. When we saw time,highly contribute to get maximum yield on the graph,particle size also affect to yield. The following residual plots were acceptable. Both the normality and uniformity of variance assumptions were verified.



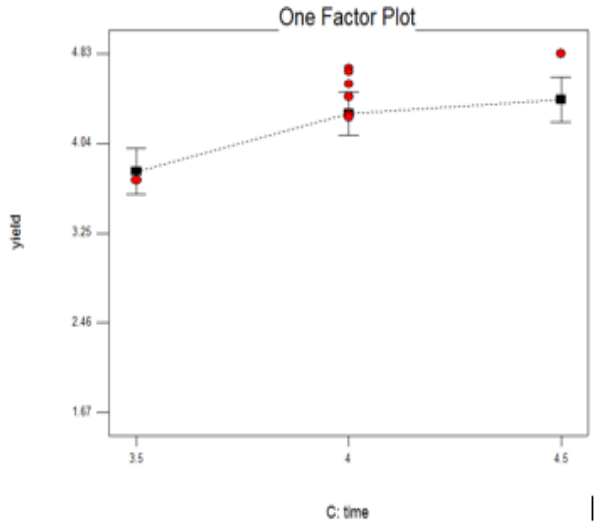
Figures 4.4: normal plot vs effect interaction and predicted vs actual graph.

The prediction value 3.308 and the actual value 4.83 from the pridiction vs with actual graph.

Normal probability graph shows highly affected the extraction,the effect were seen B,C,ABC,AC,AB,BC,A based on the above graph the list effect on the yield were seen.the yield of nigrum oil strongly affected by temperatures,the percent contribution were high when compare from the other,time also a greater factor when we compared to the other interaction factors.

The interction of paricle size,temperatures,and time also affected on the yield.

4.3.2 One Factor Effect of the Fennel oil



figures4.5:A effect of time on yield

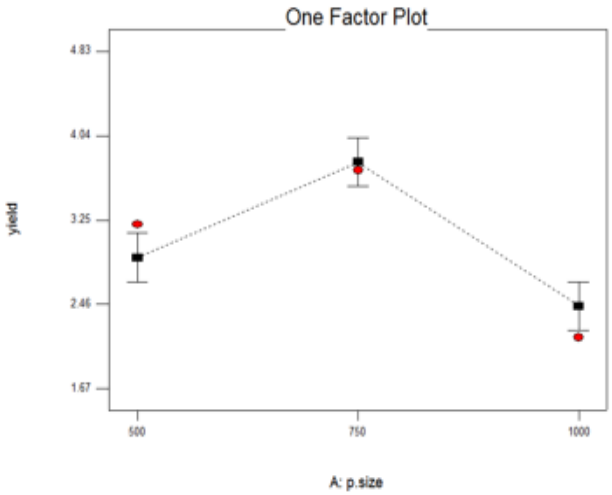


figure4.5:B effect p.size on yield.

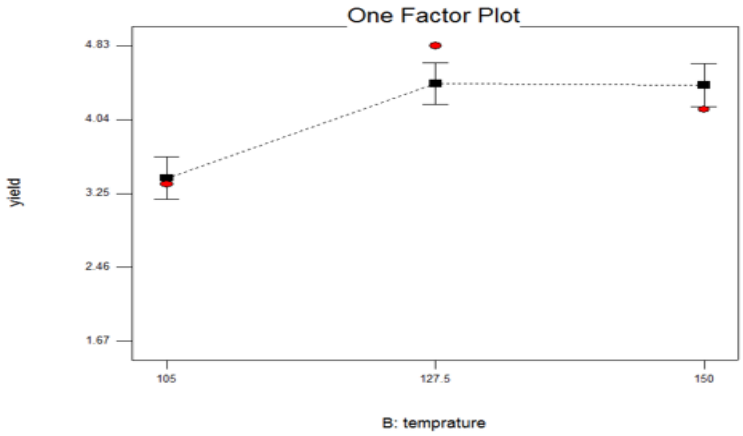


figure4.5:C effect of temperature on the yield

from the above figures A one factor plot the time for the given extraction result highly affected the yield on the particle size of 750micro.m,at the 4:30hr the high amount of yield extracted about 4.83%. in figures B the partilce size of the nigrum seed also affected the extracted oil the optimum point of the size were 750micro.m the yield also highly performed about 4.04% under the time of 3:30hr. in figures C also the maximum temperature of the distillation at the optimum point of particle size and time,the nigrum seed oil was highly extracted and amount of yield also high. at the temprature of 127.5^{o}c and time 4:30hr and p.size 750, the yield of the oil was 4.5%.

From the above graph the following stastics were seen in the main effect on the yield.

Response: yield

ANOVA for Selected Factorial Model

Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F >Value	Prob > F	
Model	7.48	7	1.07	3.56	0.0097	Significant
A	0.93	1	0.93	3.11	0.0911	
B	4.44	1	4.44	14.80	0.0008	
C	1.80	1	1.80	5.98	0.0225	
AB	0.11	1	0.11	0.37	0.5472	
AC	8.533E-003	1	8.533E-003	0.028	0.8676	
BC	0.12	1	0.12	0.41	0.5291	
ABC	0.065	1	0.065	0.22	0.6466	
Curvature	10.97	1	10.97	36.53	< 0.0001	Significant
Residual	6.91	23	0.30			
Lack of Fit	6.74	18	0.37	11.18	0.0071	significant
Pure Error	0.17	5	0.033			
Cor Total	25.36	31				

The Model F-value of 3.56 implies the model was significant. There was only a 0.97% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500

indicate model terms were significant. In this case B, C were significant model terms. Values greater than 0.1000 indicate the model terms were not significant.If there were many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Curvature F-value" of 36.53 implies there was significant curvature (as measured by difference between the average of the center points and the average of the factorial points) in the design space. There was only a 0.01% chance that a "Curvature F-value" this large could occur due to noise. The "Lack of Fit F-value" of 11.18 implies the Lack of Fit was significant. There was only a 0.71% chance that a "Lack of Fit F-value" this large could occur due to noise. Significant lack of fit was bad -- we did the model to fit.

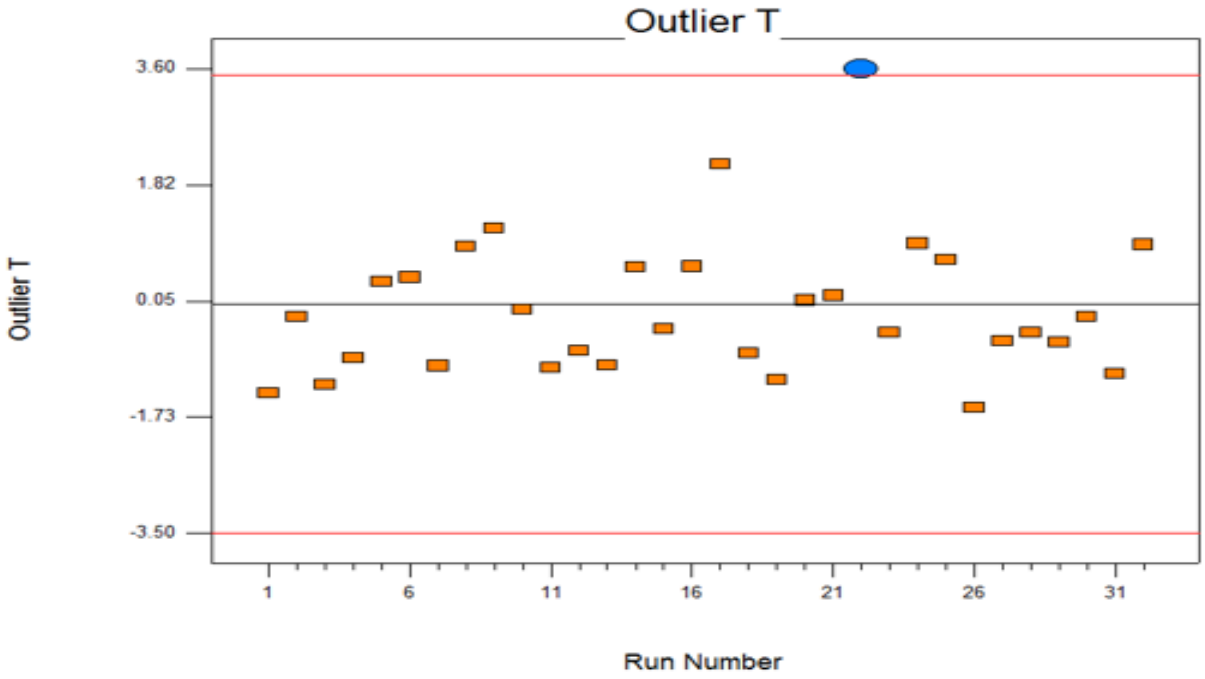


Figure 4.5: outlier vs run number graph for variance analysis.

A very common defected that often shows up on normal probability plots was one residual that was very much larger than any of the others. Such a residual was often called an outlier**.** The presence of one or more outliers had seriously distorted the analysis of variance, so when a potential outlier was located, careful investigation. From the above graph the point locations had one point those indicted the outlier had to more informative than the rest of the data.

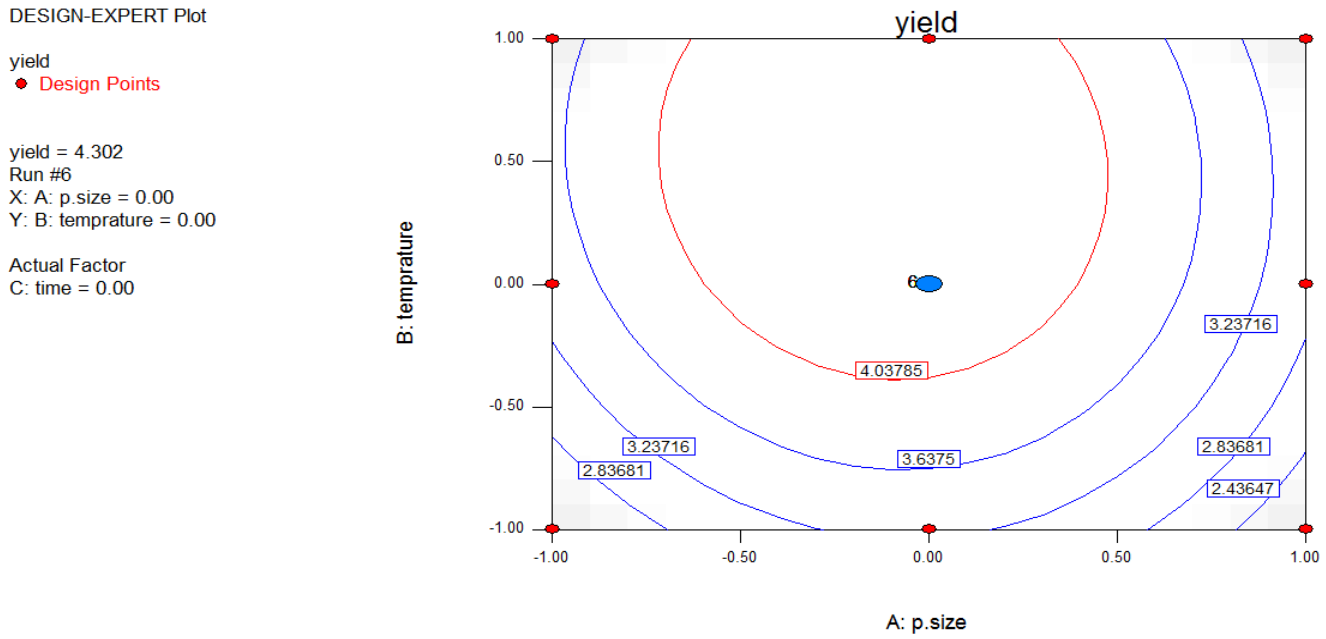


Figure 4.6: the counter graph interaction of p.size vs temperatures for optimum yield points

At the above graph the yield amount 4.302%at the p.size,temperatures,and time with center point.this yield recorded with the optimum condition.so as above 4%percent yield were high based on litrature review.

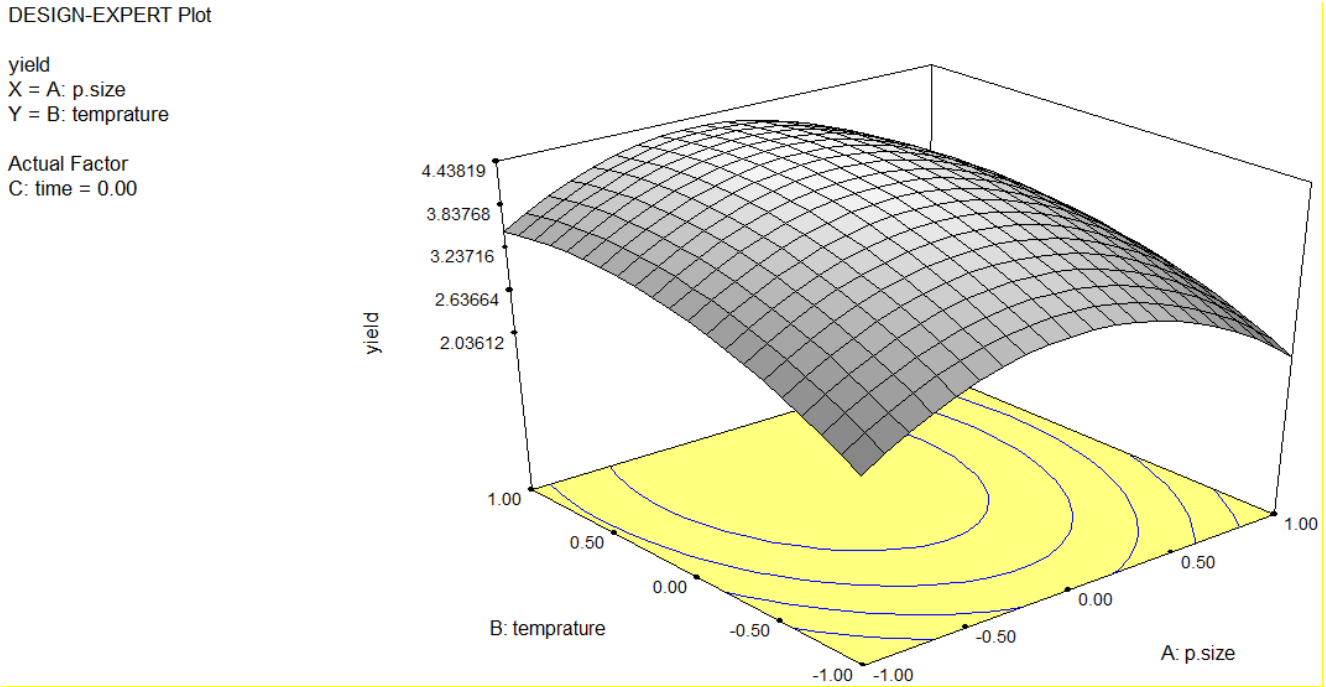


Figure 4.7: 3D surface graph with the interaction of the three factors.

3D surface graph had an advantages to investigate the three factors in one graph from the above curve. The optimum design points were observed including temperatures, particle size, and time with counter curve so the optimum points from the graph the yield content were 4.7226 with particle size 750micro.m at the temperature of 127.5^0c.

4.4 Comparison of Black Pepper Seed oil from its Leave oil

Table 4.4: For leave oil design expert using box Hicken method.

Std	Run	Block	temprature	Particle size	Time	Yield
1	16	Block 1	105	500	1.00	2.3
2	4	Block 1	150	500	1.00	3.46
3	13	Block 1	105	1000	1.00	2
4	9	Block 1	150	1000	1.00	2.25
5	8	Block 1	105	750	0.00	2.1
6	17	Block 1	150	750	0.00	2.4
7	14	Block 1	105	750	2.00	2.6
8	2	Block 1	150	750	2.00	2.7
9	11	Block 1	127.5	500	0.00	3
10	3	Block 1	127.5	1000	0.00	2.5
11	7	Block 1	127.5	500	2.00	3.4
12	5	Block 1	127.5	1000	2.00	2.9
13	15	Block 1	127.5	750	1.00	3.79
14	1	Block 1	127.5	750	1.00	3.79
15	6	Block 1	127.5	750	1.00	3.79
16	12	Block 1	127.5	750	1.00	3.79
17	10	Block 1	127.5	750	1.00	3.79

Response: yield

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	7.15	9	0.79	81.96	< 0.0001	significant
A	*0.58*	*1*	*0.58*	*59.77*	*0.0001*	
B	*0.86*	*1*	*0.86*	*88.53*	*< 0.0001*	
C	*0.29*	*1*	*0.29*	*29.65*	*0.0010*	
A^2	*3.53*	*1*	*3.53*	*364.64*	*< 0.0001*	
B^2	*1.05*	*1*	*1.05*	*108.09*	*< 0.0001*	
C^2	*0.82*	*1*	*0.82*	*84.27*	*< 0.0001*	
AB	*0.30*	*1*	*0.30*	*30.90*	*0.0009*	
AC	*9.943E-003*	*1*	*9.943E-003*	*1.03*	*0.3447*	

BC	*4.519E-006*	*1*	*4.519E-006*	*4.665E-004*	*0.9834*	
Residual	0.068	7	9.687E-003			
Lack of Fit	*0.068*	*3*	*0.023*			
Pure Error	*0.000*	*4*	*0.000*			
Cor Total	7.21	16				

The Model F-value of 81.96 implies the model was significant. There was 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 were significant. In this case A, B, C, A^2, B^2, C^2, AB were significant model terms. In this oil also temp.p.size and time were also highly significant in the process.Values greater than 0.1000 indicate the model terms were not significant. If there were many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Std. Dev.	0. 098	R-Squared	0.9906
Mean	2.97	Adj R-Squared	0.9785
C.V.	3.31	Pred R-Squared	0.8252
PRESS	1.26	Adeq Precision	23.328

The "Pred R-Squared" of 0.8252 was in reasonable agreement with the "Adj R-Squared" of 0.9785."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 was desirable. Your ratio of 23.328 indicates an adequate signal. This model could be used to navigate the design space.

Final Equation in Terms of Actual Factors:

yield=-37.36031+0.51823*tem+0.019468*size+1.3565*time-1.83778E-003*tem^2

-9.89048E-006*$size^2$-0.44042*$time^2$-4.66606E-005*tem * size-2.20959E-003*tem*time

+4.09184E-006* size * time.

The yield of leave oil identified below with counter graph.

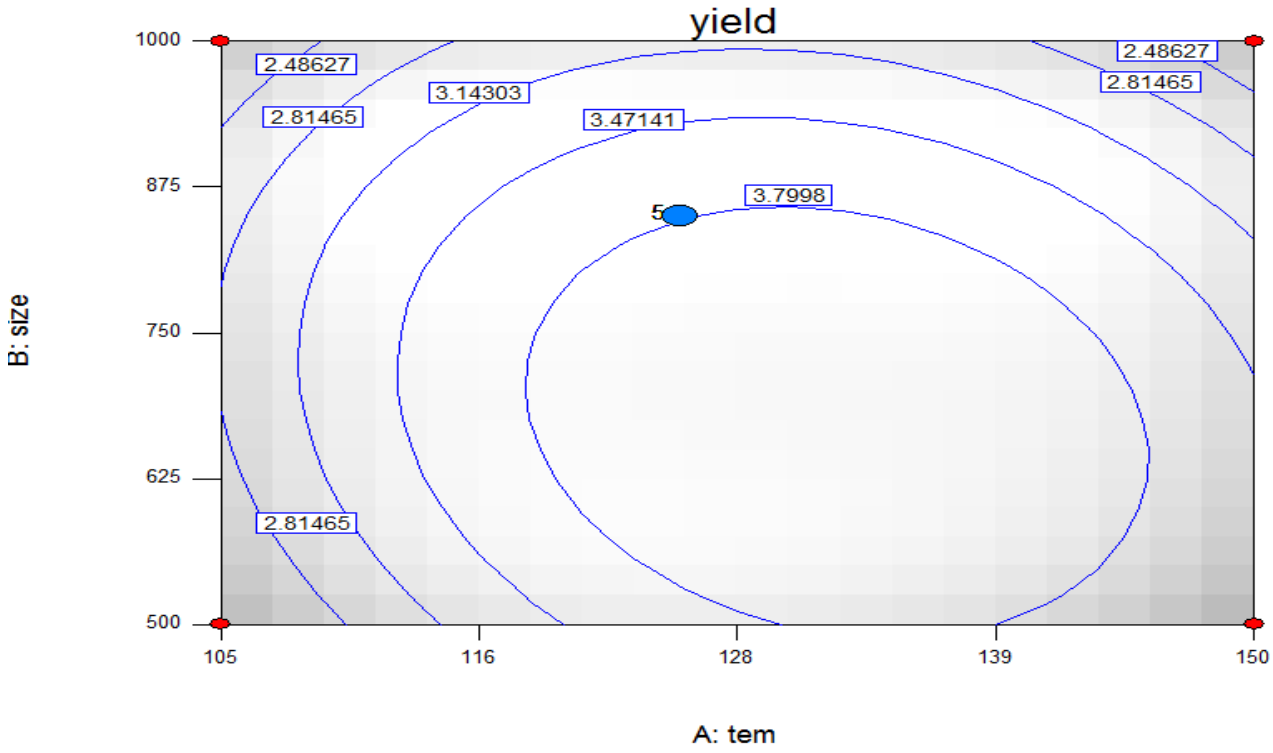
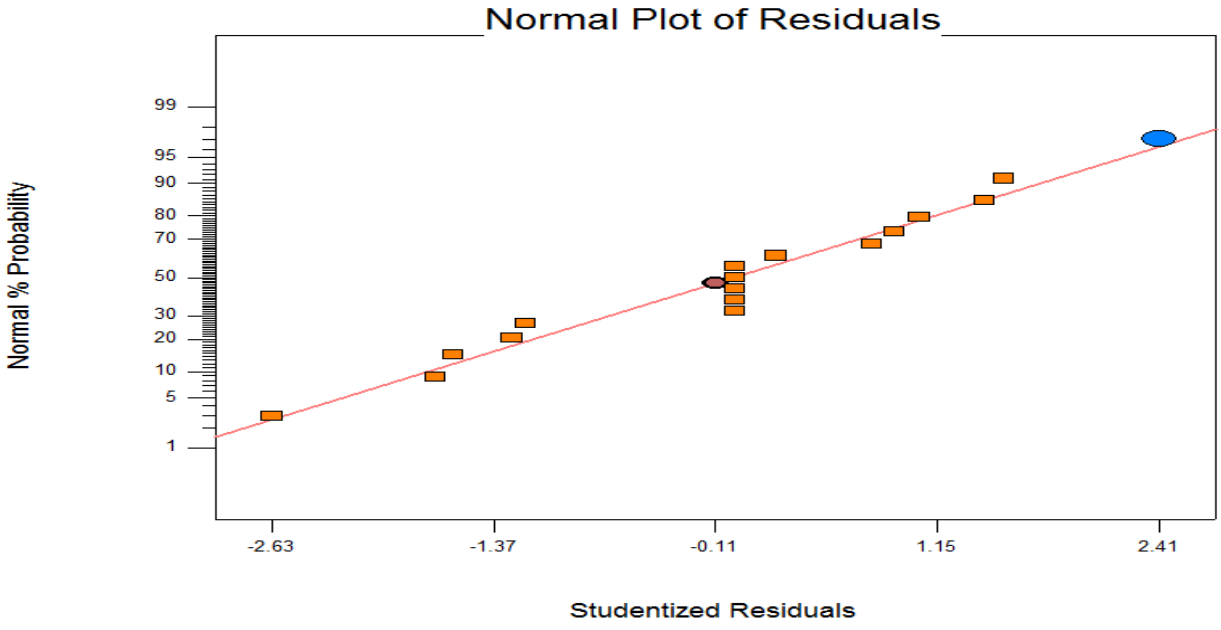


Figure 4.8: counter graph of interaction temperatures vs p.size of leave oil

Temp127.5 and particle size 750,time 4:00hr.run order #15 yield 3.79.from the counter graph the 5th curve indicates the design space from the counter graph. The leave oil tempratures and seed oil tempratures almost the same from the graph. The yield also satisfactory when compared to the seed oil.to accept the yield of oil compartions were necessaries.



The point indictes maximum point std order 2 and residuals .2.41 and y= probablility 97.1 %.probablity graph more advantages.

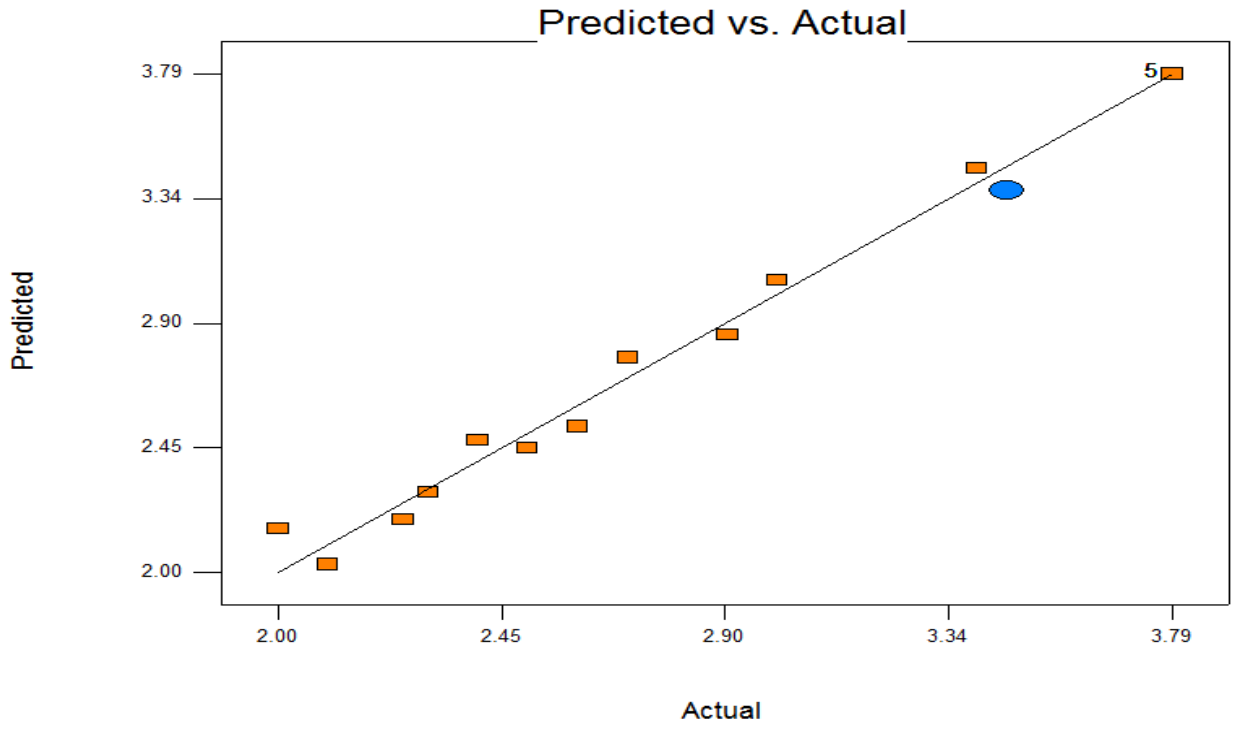
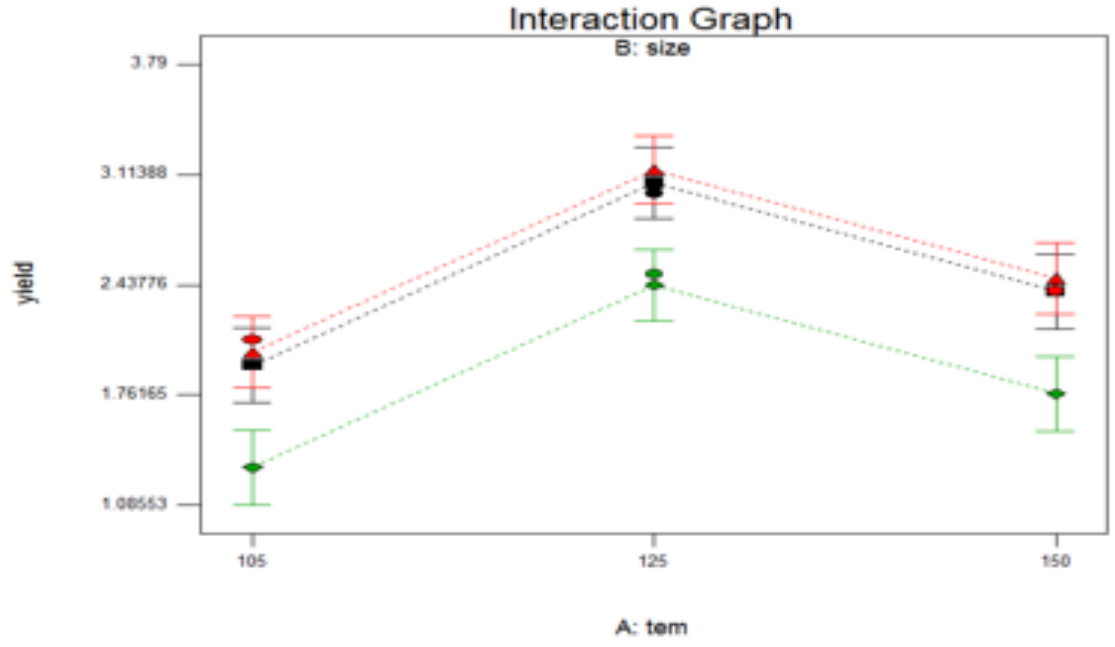


Figure 4.9:normal plot resduals and studentized and predicted vs actual value

X:actual =3.46, and y: predicted value 3.372 at std no 2,the graph result were satisfied.

Figure 4.10: interaction of temperatures and p.size to yield.



From the above all of one factor plot the optimum design point were located on the graph but particle size and temperatures were highly affected the design points. The design point were minimum 500, 750, 1000 time 0=all factors were coded. We summarize the difference and the similarity of the piper Nigrum seed oil and its leave oil, the yield of both oils had similar content instead of distillation time a little difference. The maximum yield of leave oil 3.79 and the yield of seed oil 4.83 I checked the error some difference.4.83-3.79=1.04% difference.so our comparison for the application and characterization I selected the seed oil for my analysis in case of maximum yield.

The comparison of both nigrum seed and leave oils yield:

The amount of yield from black pepper seed oil at optimum condition (at the temp, 125, time 4:30hr and 850micro meter) from run#22 and STD 46 were 4.83 or19.2ml (12.075gm) of oil extracted by using steam distillation when compared to the literature value the error should below calculated. The same thing the amount of leave oil would be 3.79%.

True error%= (standard value-experimental value/standard value)*100…… eqn (4.2)

From the literature value the maximum yield of piper nigrum seed and leave oil were 6.12%and 5% respectively.so as I calculated the error as follows. So true error for the seed oil= $trueerror = \frac{6.12 - 4.83}{6.12} * 100\% = 21.078\%$, so the remaining

Percent=78.92%would the amount of yield successful but some limitation,

Error from the leave oil $= trueerror = \frac{5 - 3.79}{5} * 100\% = 24.2\%$.The mechanism of both leave and seed oil extraction method were similar steam distillation for the quality of oil, but it affected the amount or the yield. From true error percentage it acceptable according to literature review percent, the yield content depends on the raw quality and the lab equipment. The comparison of leave and seed oil error were almost some variation. This indicates we use leave oil as substitute of seed oil.

4.5 Optimization of Process Variables

The piper nigrum oil had been extracted with steam distillation to get clear and quality of essential oil.

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
p.sizeis	in rang	500	1000	1	1	3
temperature	in range	105	150	1	1	3
time	maximize	3.5	4.5	1	1	3
yield	maximize	1.67	4.83	1	1	3

Solutions

Number	p.size	temprature	time	yield	Desirability	
1	723.35	136.36	4.50	4.5152	0.949	Selected
2	719.16	135.81	4.50	4.51451	0.949	
3	723.86	135.54	4.50	4.51448	0.949	
4	717.15	133.49	4.50	4.50566	0.947	
5	758.70	128.86	4.50	4.43988	0.936	
6	734.96	145.22	4.50	4.43182	0.935	

The results above had shown that the three process variables and the interaction among the variables affected the yield of the oil. Therefore, the next step was to optimize the process variables in order to obtain the highest amount of oil by using the model regression developed. So in order to obtain the optimum yield of oil, the predicted combination of parameters was as follows: temperature of 136^{o}c the particle size, 723.35micro.m and time 4:30hr. Under these conditions, the yield predicted of 4.51% with a desirability value of 0.949. To validate the optimum conditions predicted by the model using desirability ramp, triplicate experiments were conducted using the optimized process conditions and mean percentage value of yield 4.2% was obtained and the experiment results were related with the data obtained from optimization analysis using desirability functions. Therefore, the study shows that black pepper seed oils can definitely be used for skin care cosmetics with this optimum variables.

4.6 Fourier Transform Infra-Red (FTIR) Spectroscopy

The following FTIR result were done in Addis Ababa university faculty of chemistry.

The peaks of the curve were indicates the functional groups that exist. When we saw the peaks there were different component displayed. The graphs curve made with frequency vs to transmittance. The point of the curve reads with reader in the origin lab.

The peak of the FTIR result curve were stretching from 4000-400.the stretching of the curve difficulty to ready easily because the peaks were not sharp to identify which peaks including the functional groups.so the gridlines were mostly reduce the case of complex.

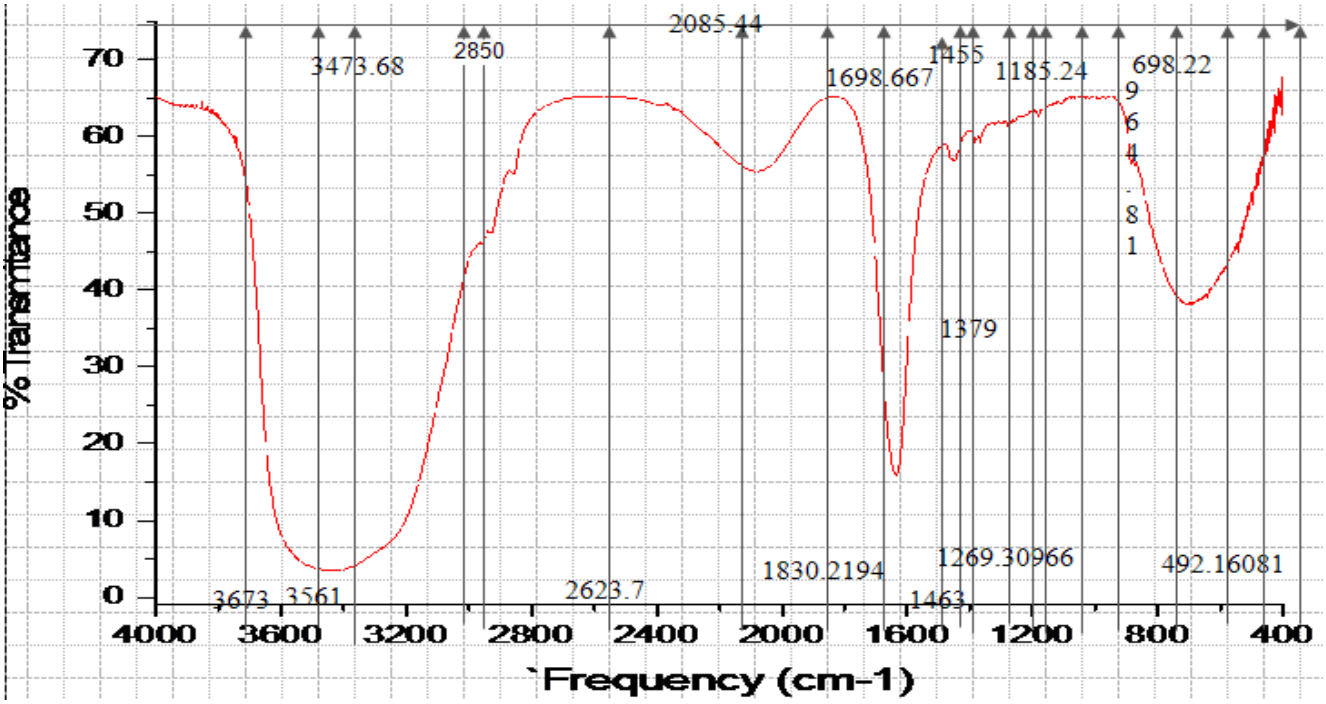
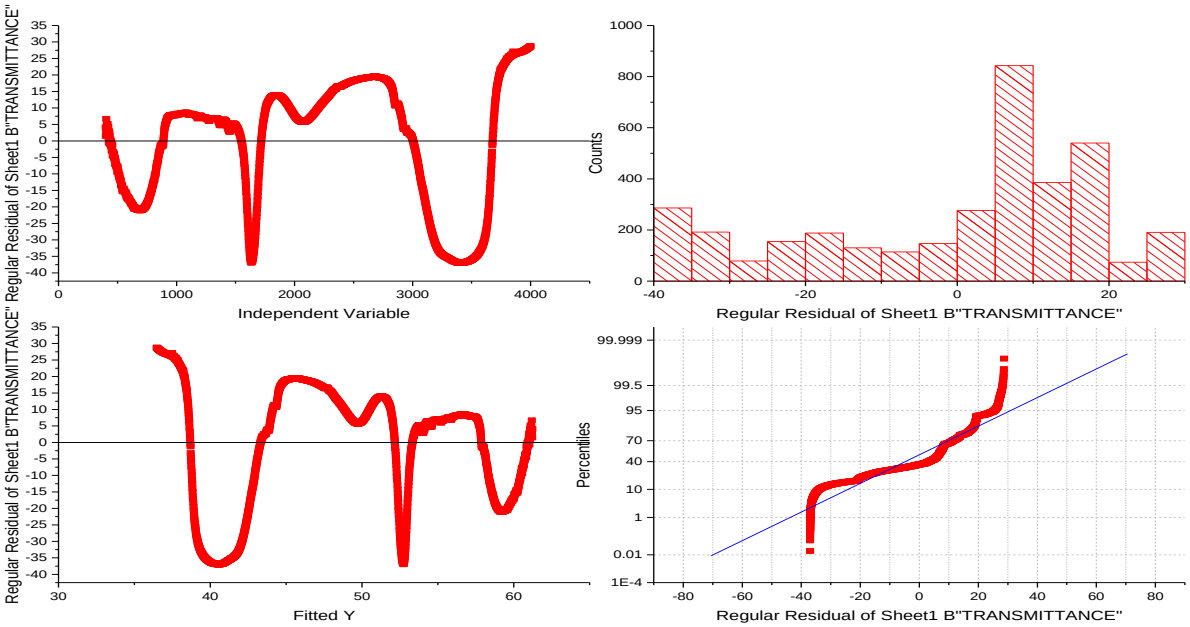


Figure 4.11.: FTIR graph analysis of functional group result

From the above graph based on the peaks the functional groups the unsaturated fatty acids c=c stretches, The O=C–H stretches in both aldehydes in the region 2830-2695 cm-1, the carbonyl stretch C=O of a carboxylic acid appears as an intense band from 1760-1690 cm-1. N–H stretch 3400-3250 cm-1.1° amine: two bands from 3400-3300 and 3330-3250 cm-1. 2° amine: one band from 3350-3310 cm-1. 3° amine: no bands in this region. N–H bend (primary amines only) from 1690-1715 cm-1, R2C=CH2 Acids, fatty acids (aromatic amines) from 1335-1250 cm-1,C–N stretch (aliphatic amines) from 1250–1020 cm-1.N–H wag (primary and secondary amines only) from 910-665 cm-1.The region from 900-650 cm-1. Aromatics, alkyl halides, carboxylic acids, amines, and amides show moderate or strong absorption bands (bending vibrations) in this region. All spectra contains peaks attributable to unsaturated fatty acids in 1690 cm−1 – 1715 and peaks attributable to unsaturated C-H asymmetric stretching for fatty acids, which shows the acidity of the oil highly concentrated (Sharma et al. 2004; Xing et al. 2007).

The linear trace of the FTIR result would magnify the frequency in the long way with its transmittance.



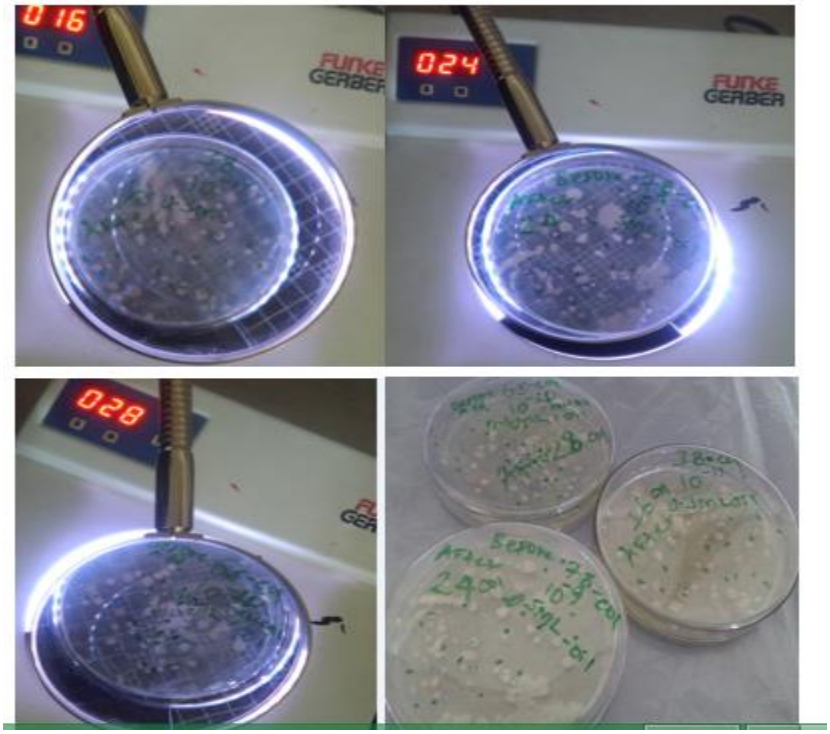
Figures 4.11.2: the transmittance and residual graph with dependent and independent variables.

The component from the functional groups were depends on the factors or chemicals that would too added into the samples, in my samples added NaCl to detected in the FTIR machine, the frequency and absorbance were highly dependent among to the result. The dependent variables were affected to the value or affected to the curve on the graph.

4.7 Anti –bacterial properties of Black Pepper oil

The oil had the anti -microorganism properties, one of this micro spices were bacterial cell, the bacterial cell should be specified under gram positive and gram negative spices.in my work gram positive bacterial spices were identified because this spices attack our skin but not all bacteria attack our body. The bacterial growth and culture were depend on the bacterial spices and the conditions to develop in suitable manner. Under gram positive spices, staphylococcus aureus specified, because this spices are affect our skin and our body.

The cultured bacterial cell were counted after that added piper nigrum oil into the petri dish after 24hrs the effect of the bacterial colonies were below:



Figures 4.12: the number of bacterial colonies after addition of oils

The following colonies were counted from 10^{-11}, 10^{-10}, 10^{-9} were 38, 65, 78 rescpectively.

From the first my selection petri dish bacteria cell=$38/10^{-11}=38*10^{11}$bactrial cell were counted. From the second my selection= $65/10^{-10}=65*10^{10}$ bacterial cells were counted.

3rd selection=$78/^{10-9}$ =$78*10^{9}$bactrial cells were counted. This colonies were counted before added of my samples.

The main objectives had not count this colonies my purpose should be after added the oil what was the effect of oil for the inhibition of bacterial growth. Then after added my oil by using sterile transfer pipette measured the oil and added into the petri dish for the following 24hr at the same temperature. The following figure and tables given the information in detail about the amount of oil added into the petri dish the effect of the essential oil. The added oil into petri dish based on literature review from 0.25-5ml of oil, so according to this value, I selected from 0.3ml-0.5ml of seed oil added for my investigation. In this case there was some difficulties to added into the petri

dish the amount of colonies that cultured were one problem.in my standing point little amount of colonies countered to little amount oil were added the small added oil into small no of colonies were inhabit the bacterial cell for to become we concluded the enough added oil into small amount to become efficient. So I added small amount of oil for small number of colonies.

Table 4.5: bacterial cell difference before addition of oil and after addition of oil

Petri dish sample and colonies	Amount of oil added into petri dish	After added the oil the remaining no.colonies	Bacterial cell counted after addition
10^{-11}, 38	0.3ml	16	$16*10^{11}$
10^{-10}, 65	0.4ml	24	$24*10^{10}$
10^{-9}, 78	0.5ml	28	$28*10^{9}$
10^{-8},135	0.5ml of leave oil	53	$53*10^{8}$

The difference of bacterial cell before and after was the following

From the first petri dish the difference after adding my sample was = $(38-16)*10^{11}=22*10^{11}$

From 2nd the difference = $(65-24)*10^{10}$ $=41*10^{10}$, and the third petri dish in my selection = $(78-28)*10^{9}$ $=50*10^{9}$

Sample and colonies	Bacterial cell counted after addition	The missed or injured cell after addition
10^{-11},38	$16*10^{11}$	$22*10^{11}$
10^{-10},65	$24*10^{10}$	$41*10^{10}$
10^{-9},78	$28*10^{9}$	$50*10^{9}$

To select the best amount of addition of oil into the petri dish by taking the ratio:

The ratio of bacterial cell or colonies missed or injured after addition/ total bacterial cell or colonies counted gives [22/38]*100=57.9% bacterial cell would be missed by addition of 0.3ml of seed oil into the petri dish. This indicates the oil affected the growth of bacteria. When we saw the second petri dish from my selection the amount of colonies defeated from the total one was= [41/65]*100%=63.077% colonies or cell was injured by adding 0.4 ml of oil, from 3rd one[50/78]*100=64.1% would be missed from petri dish by added 0.5ml of oil. When we compared from the three the third one was efficient. For the leave oil I used $10^{-8}$from the petri dish before added the no of colonies 135, counted and cells were $135*10^{8}$ after addition of 0.5ml of leave oil it became $53*10^{8}$ cells counted the missed or injured cells were 82 colonies when I calculated the ratio become 60.074%, so as to concluded the leave oil and seed oil anti -bacterial effect were almost the same.

4.8 Acidity properties of the Black Pepper Seed Oil

After did all things I checked the acidity of the essential piper nigrum seed oil.

Took 10 gm or 16ml of piper nigrum oil mixed in accurately weighed by using 60^{o}c temp, in 16 ml mixture of equal volume of alcohol but the alcohol concentration 97% and solvent hexane, the flask was connected to reflux condenser and slowly heated, until sample was dissolved completely, to this three drop of phenolphthalein added and titrated with 0.1N NaOH, until the color changed pink color appeared after shake for 30 seconds. I performed three run to select the best result.

From the first experiment, the color was changed into pink color by shaking the flask at 9.45 ml of NaOH, the acid value at the first run 5.3.The next run, the color also changed into pink color at titration of 10.16ml of NaOH consumed. The acid value would become.5.7.

3rd experiment, at the same procedure the color changed into pink with the consumption of 9.7ml of NaOH the acid value 5.57.

Exp.no	Amount of 0.1N NaOH	Amount of oil	Acid value
1	9.45ml	16ml or 10gm	5.3
2	10.16ml	10gm or 16ml	5.7
3	9.7ml	16ml	5.57

From the above three result I took the average value. The acid value were 5.5.From this I concluded the piper nigrum oil had any admirable quality or attribute as skin care application. The essential oil of the nigrum oil acidity value not much acidic it's nearest to human being acid value.

4.9 GC-MS /Gas Chromatography Mass Spectroscopy

The GC-MS result experiment were done in the leather industry development institute in Addis Ababa akaki kality sub city. For GC-MS analysis the samples were taken from optimum conditions extraction. The oil samples were taken from the factors of temperature, time and particle size, 127.5^{o}c, 4:30hr, and 750micro.m respectively. All result were identified below with the help of graphical data.

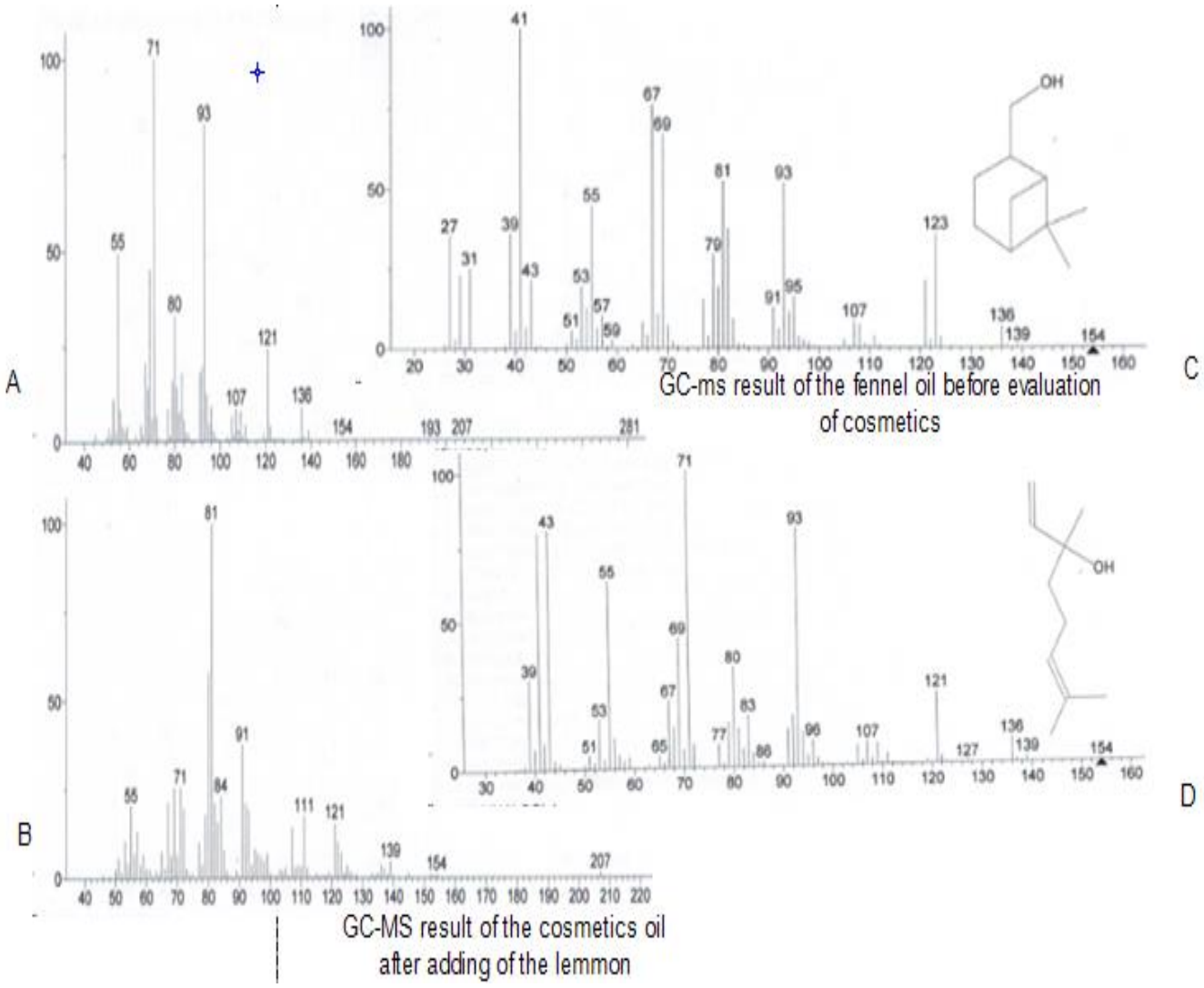


Figure 4.13 GC-MS graphical result of the composition

From the above scanned graph data the following compounds were identified. the compound was wrote below indicates the main ingredients of the cosmetics oil.

Table 4.6: GC-MS result of the composition percent

No.	Component	Pk	Peak code	Compositions with area%
1	1,2,3-propanetriol,glycerol,glyrol&glycerin, Osmoglyn	81	81999	0.91
2	Caparratriene,1,2,4-trimethyl cyclohexane	80	80579	0.20
3	Longifolen aldehyde	84	84231	2.21
4	4H-pyran-4-one,3-hydroxy-2-methyl,maltol	71	71256	0.19
5	2-ethyl-4-methyl-5,6-dihydro-2H-py	69	69255	0.19
6	Guaidiol	67	67214	0.22

From the above graph of A and C Longifolen aldehyde 2.21% had highest percent composition. The 1, 2, 3-propanetriol, glycerol, glycerol& glycerin .91%, Osmoglyn were exist in this oil indicates the oil used as for cosmetics, the glycerol and glycerin also used for an antiseptic. The compound formula of glycerin $C_3H_5(OH)_3$ it's also combined with various acids, as oleic, margaric etc. and also called as glycerol. From the result of piper nigrum seed oil I concluded, it used for cosmetics due to the existence of this compound.

From the graph of B and D the following compounds were displayed after preparation of skin care cosmetics.

No	Component	Pk	Peak code	Composition area %c
1	(1R,5S,E)-2-methyl-4-[2,2,3-trimethyl-6-methylidenecyclohex-2-en-1-yl]but-2-enal	71	71999	0.80
2	Cis-13-octadecenoic acid	39	39303	13.33
3	Alpha, -terpineol	55	55633	3.69
4	(P-MENTHA-4,8-DIENE,5-METHYL-	67	67228	0.14

	),CYCLOHEXENE,1-ISOPROPENYL-2,4-DIMETHYL			
5	A-PHELLANDRENE EPOXIDE	69	69438	0.36
6	Oleic acid	80	80337	21.94
7	Ledene oxide	93	93799	22.54

From the above GC-MS result the amount of oleic acid, Ledene oxide were high amount of composition area covered. From the result the percentage of oleic acid 21.94% high composition and also Ledene oxide 22.54%. Above 180 component were displayed. When we saw the component composition the maximum percent covered by unsaturated fatty acids, alcohols, and phenolic compounds. From the above GC_MS result oleic acid were one of the skin care oil compound. 9, 12-octadecadienoic acid [Z, Z], Octadecanoic acid, 9-octadecenoic acid,(E)-cis-13-octadecenoic acid 13.33%, n--Hexadecanoic acid 8.61% , 4,4 ,8-trimethyltricyclo[6.3.1.0(1,5) dodecane-2,9-diol,5-pyrrolidin-2-ylidenemethyl-3,4-dihydropyrrol-2-one,ledene oxide-(I) 3.61%, (z)-2,6,6-trimethyl-.alpha.-(1-propenyl)-2-cyclohexane-1-methanol,4-(1,3-dimethyl-3-cyclohexanyl)-1 3.69%,6-heptadien-4-ol , were high percent covered.so as I concluded the oil skin care cosmetics were money advantages for human being, specially body care, injured body care and infected body due to the existence of the oleic acid and other unsaturated fatty acid.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

This study supplies useful information for optimization and characterization of the process of steam extraction of piper nigrum seeds. The highest yield of total extract and the highest content of the target compounds with anti-bacterial, properties was obtained from optimum essential oil temperature. The minimum amount of liquid (ethanol) and distilled water solvent ration were 1:2, and the solid to liquid ration 1:3. The minimum amount of oil from the result of extraction 1.67gm or 6.63ml of oil at which solvent concentration were not much diluted and the operation temperature nearest to water boiling temperatures. The raw material was 3 times less than the amount of the extraction solvent material. The optimal process duration was about 4:30hr. Longer contact time did not lead to a better yield above this hours. The piper nigrum oil anti -bacterial properties were above 60% efficient, the particle size of the optimum yield 750micro meter.

The characterization of the samples were by the FTIR, GC_MS analysis. The samples were took from the optimum condition extracted oil from the temperature of 127.5^{o}c, particle size 750micro.m and time of 4:30hr.

The GC_Ms analysis result were mostly unsaturated fatty acids from this oleic acid, linalool, hexandoxic acid, n-Hexadecanoic acid, Octadecanoic acid, and for the optimization of process variables the result were closely related with model, the optimized value of yield of the oil 4.2%.

Generally the oil had some application such as skin care, pharmaceuticals, and condiments for additives and for bacterial infection body by directly apply to the infected body.

5.2 RECOMMENDATIONS

The piper nigrum oil one of the parts of small yield product of essentials. The black pepper parts of body have to be different application from its stems, leaves, and seed,

The oil source of raw materials were locally cultivated there was not done awareness for the farmers, so for the future work the farmer planting systems shall change to modernization for the availability and regular supply of the seeds for commercial application.

Based on the result of the GC-MS many compounds were identified, from the components the unsaturated fatty acids are highly concentrated in the oil, so this is the signs of skin care cosmetics and pharmacies product of oil, the oil anti -bacterial properties had been a good enough from the result test of s. aureus experiments, from the lab result its compositions are highly determinant for very common merit. For future work this extraction helps for anyone who study's in this area, can use as a base. I recommend for the cosmetics company, they used as additives for large scale application such as skin care cosmetics and pharmaceutical product.

In this research, there was studied of process variables optimization, for future study this extraction helps for optimization with cost supply of the oil.

In spite of this the highly alcohol and phenol content of this oil can use for human injured body by directly smear to the injured parts of the body, the pharmacicual industrial company can used for as an additives for production of anti-microbial drug preparation.

6. REFERENCES

Acimovic, M., Tesevic, V., Todosijevic, M., Djisalov, J., & Oljaca, S. (2015). Compositional characteristics of the essential oil of Pimpinella anisum and Foeniculum vulgare grown in Serbia, *39*(1), 9–14.

Afiq, A., Rahman, A., & Man, C. (2013). Date seed and date seed oil 1, *20*(5), 2035–2043.

Angelov, G., & Boyadzhieva, S. (2016). Extraction of Fennel (Foeniculum vulgare) Seeds : Process Optimization and Antioxidant Capacity of the Extracts (Vol. 30, pp. 245–253). https://doi.org/10.15255/CABEQ.2015.2281

Badgujar, S. B., Patel, V. V, & Bandivdekar, A. H. (2014). Foeniculum vulgare Mill : A Review of Its Botany , Phytochemistry , Pharmacology , Contemporary Application , and Toxicology, *2014*.

Berk, M., & Zielke, T. (2013). Steam distillation of fennel oil from fennel seed, 1–5.

Bernardo, F. P., & Saraiva, P. M. (2005). Integrated Process and Product Design Optimization : a Cosmetic Emulsion Application.

Bowes, K. M., & Zheljazkov, V. D. (2005). Essential Oil Yields and Quality of Fennel Grown in Nova Scotia, *39*(7), 1640–1643.

Brazil, S. P. (2006). Essential oil constituents of different organs of fennel (Foeniculum vulgare var . vulgare)., 193–198.

Butler, H. (n.d.). *Poucher â€™ s Perfumes , Cosmetics and Soaps Edited by*.

Coelho, J. P., Cristino, A. F., Matos, P. G., Rauter, A. P., Nobre, B. P., Mendes, R. L., … Palavra, A. F. (2012). Extraction of Volatile Oil from Aromatic Plants with Supercritical Carbon Dioxide: Experiments and Modeling, 10550–10573. https://doi.org/10.3390/molecules170910550

Composition, C., & Cultivars, F. (2011). Chemical Composition, Antimicrobial and Antioxidant Activities of Essential Oils from Organically Cultivated Fennel Cultivars, 1366–1377. https://doi.org/10.3390/molecules16021366

Cosmetics, A., Products, H. B., Products, M., Cosmetics, S. C., Care, B., Care, H., … Creams, H. (n.d.). Herbal Cosmetics.

Damayanti, A., & Setyawan, E. (2012a). Essential Oil Extraction of Fennel Seed (Foeniculum vulgare) Using Steam Distillation, *3*(October), 12–14.

Damayanti, A., & Setyawan, E. (2012b). *Essential Oil Extraction of Fennel Seed (Foeniculum vulgare) Using Steam Distillation*. *International Journal of Science and Engineering* (Vol. 3). https://doi.org/10.12777/ijse.3.2.12-14

Dreger, M., & Wielgus, K. (2013). Application of essential oils as natural cosmetic preservatives, *59*(4). https://doi.org/10.2478/hepo-2013-0030

Dua, A., Garg, G., Mahajan, R., & Dua, A. (2013). Polyphenols , flavonoids and antimicrobial properties of methanolic extract of fennel (Foeniculum vulgare Miller), *3*(4), 203–208.

English, D. J., Manager, S., & Products, A. (n.d.). Microbiology in Cosmetics – Challenges in Cosmetic Manufacturing Drug vs . Cosmetic, 1–85.

Essential Oils : From Conventional to Green Extraction. (n.d.), 9–21. https://doi.org/10.1007/978-3-319-08449-7

Fennel Benefits & Information (Foeniculum Vulgare). (n.d.).

Format, S., Isbn, P., Pacific, A., Press, B., & Indian, T. (n.d.). Handbook on Cosmetics (Processes , Formulae with Testing Methods), 1–11.

Gediya, S. K., Mistry, R. B., Patel, U. K., Blessy, M., & Jain, H. N. (2011). Herbal Plants : Used as a cosmetics, *1*(1), 24–32.

Gulfraz, M., Mehmood, S., Minhas, N., Jabeen, N., & Kausar, R. (2015). Composition and antimicrobial properties of essential oil of Foeniculum vulgare Composition and antimicrobial properties of essential oil of Foeniculum vulgare, (October). https://doi.org/10.5897/AJB08.715

Hans, J. (2011). Extraction of Natural Products from Plants – An Introduction, 1–26.

Hassan, E. A., Hamad, E. H., & Departments, H. (2009). The Chemical Constituent and Vegetative and Yielding Characteristics of Fennel Plants Treated with Organic and Bio-fertilizer Instead of Mineral Fertilizer, *3*(2), 579–587.

Hou, X., & Jones, B. T. (2000). Inductively Coupled Plasma / Optical Emission Spectrometry, 9468–9485.

Hyde, K. D., & Bahkali, A. (2010). Fungi - An unusual source for cosmetics, (July). https://doi.org/10.1007/s13225-010-0043-3

Kooti, W., Moradi, M., Ali-akbari, S., Sharafi-ahvazi, N., Asadi-samani, M., & Mill, F. (2015). Therapeutic and pharmacological potential of Foeniculum vulgare Mill : a review, *4*(1), 1–9

Liu, K. (2009). Bioresource Technology Effects of particle size distribution , compositional and color properties of ground corn on quality of distillers dried grains with solubles (DDGS). *BioresourceTechnology*, *100*(19), 4433–4440.https://doi.org/10.1016/j.biortech.2009.02.067

Metabolites, S. (2011). Essential Oils from Steam Distillation, 1–9.

Ministry, M., Welfare, F., Food, N., Mission, S., Revolution, B. G., Programme, C. D., … View, A. (2016). Wo o dh ea d Pu bl in g ite d, (January 2012).

Ministry, M., Welfare, F., & View, H. (2016). Wo o dh ea d Pu bl in g ite d, (January 2012).

Mohammadi, S., & Aminifard, M. H. (2013). essential oil of fennel (Foeniculum vulgare L .), *14*(1), 420–431. https://doi.org/10.5513/JCEA01/14.1.1217

one, p., principle, o., & ftir, o. f. (n.d.). fourier transform infra-red (FTIR) spectroscopy theory and operation principle of ftir spectrometer, 1–12.

Part, N., Botanical, L., Lemongrass, U., Cymbopogon, E., Citronella, C., Cineale, T., … Seed, N. (n.d.). SMALL-SCALE PRODUCTION, 1–10.

Q, M. J., & Flora, E. W. B. (n.d.). Production of Essential Oil from Lemongrass.

Rozalli, M., H, N., L, N., & A, Y. (2014). Particle Size Distribution of Natural Peanut Butter and its Dynamic Rheological Properties, (2010).

Sarkic, A., & Stappen, I. (2018). Essential Oils and Their Single Compounds in Cosmetics — A Critical Review, 1–21. https://doi.org/10.3390/cosmetics5010011

Seed productivity , yield and composition of the essential oil of fennel Foeniculum vulgare var . dulcis in the season of the year. (2006), (1992), 86–90.

Service, U. S. C., & Kong, H. (2015). Cosmetics & Toiletries Market Overviews 2015.

Shin, Y., Jung, M., Kim, N., Kim, K., & Lee, D. (2007). Characterization of Fennel Flavors by Solid Phase Trapping-Solvent Extraction and Gas Chromatography-Mass Spectrometry, *28*(12).

Sienkiewicz, M., Sienkiewicz, M., Denys, P., & Kowalczyk, E. (2015). Antibacterial and immunostimulatory effect of essential oils Antibacterial and immunostimulatory effect of essential oils, (December).

Sumit, K., Vivek, S., Sujata, S., & Ashish, B. (2012). Herbal Cosmetics : Used for Skin and Hair Herbal Cosmetics : Used for Skin and Hair, (May 2014).

Sumit, K., Vivek, S., Sujata, S., & Ashish, B. (2014). Herbal Cosmetics : Used for Skin and Hair Herbal Cosmetics : Used for Skin and Hair, (May).

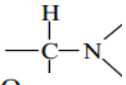
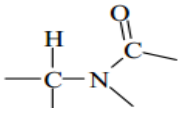
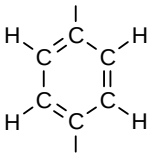
Unal, H., Izli, N., Kacar, O., & Goksu, E. (2013). Physical and nutritional properties of fennel seed, *11*(October).

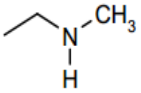
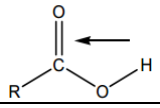
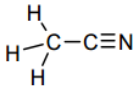
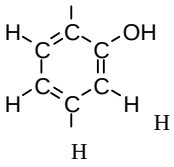
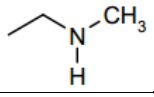
Wanjari, N., & Waghmare, J. (2015). A Review on Latest Trend of Cosmetics-Cosmeceuticals, *4*(May), 45–51.

7. APPENDICES

Appendix A: FTIR Laboratory Results

Table A-1: FTIR Laboratory Results of Functional Groups

Peak Frequency result in (cm-1) from the graph	Transmittance%	Absorbance	Range of frequency (cm-1)	result of Functional groups from the curve
492.16081	65.31	0.185	600-485	alkyl halides
				C-I stretch
698.22	38.02	0.4202	700-610 cm-1, 698.22 900-675 cm-1 730-770	–C≡C–H, C–H bend, alkyne , alkyl halides, carboxylic acids, and  amines, amides  Aromatics, Mono substituted aromatic,  H
964.81	65.0129	0.187	~966	Unsaturated fatty acids, olenic,omega-3 and linoleic fatty acids
			1000-750	Sulfonates, S-O stretch
1185.24	62.8	0.202	1210-1140	phosphine oxides, P=O
			1200-1025	Amines, C-N Stretch (alkyl)
1269.30966	61.1	0.214	1360-1250	Amines, C-N Stretch (aryl)
1320	53.063	0.275	1390-1300	nitro groups, -NO2 (aliphatic)

			1355-1315	nitro groups, -NO2 (aromatic)
1379	59.66	0.2243	1550-1370	nitro groups, -NO2, RNO2
1455	56.23	0.25	1550-1450	Amines—Secondary.  N-H Bend, N-Methyl ethylamine,
1463	57.23	0.242	1550- 1370	C–C stretch (in-ring) aromatics
1698.667	15.9955	0.796	1690-1715 1600-1675	R2C=CH2 Acids, fatty acids, unsaturated and Aromatics. 
1830.2194	65.05	0.1867	1830-1800	Anhydrides, C=O stretch
2077.23	55.56	0.255	2077.23~2100	Alkyne, H CCCH$_3$
2085.44	55.5	0.254	2085.44~2100	Alkyne, H CCCH$_3$
2300.5	63.67	0.1960	2300-2200	Nitriles Methane nitrile 
2623.7	65	0.187	3000-2500	O-H (carboxylic acids) 
3448	3.548	1.45	3500-3200 cm-1	Phenol, and alcohol  Phenol
3473.68	3.372	1.472	3500-3200 cm-1	Phenol, alcohol, Hydrogen bonding
			3500-3300	Amines N-H stretch (1 per N-H bond) 
			3500-3180	Amides, N-H stretch

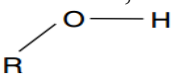
				 Methane amide 
3561	8	1.097	3300 – 3600	O-H (alcohol)
3673	9	1.045	(3700−3600 cm−1	Alcohols, O−H stretching, 

Table A-2 major component of the cosmetics oil

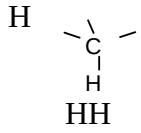
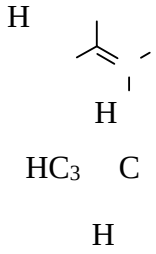
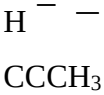
No	Component	Pk	Peak code	Composition area %c
1	(1R,5S,E)-2-methyl-4-[2,2,3-trimethyl-6-methylidenecyclohex-2-en-1-yl]but-2-enal	71	71999	.80
2	Cis-13-octadecenoic acid	39	39303	13.33
3	Alpha, -terpineol	55	55633	3.69
4	(P-MENTHA-4,8-DIENE,5-METHYL-),CYCLOHEXENE,1-ISOPROPENYL-2,4-DIMETHYL	67	67228	.14
5	A-PHELLANDRENE EPOXIDE	69	69438	.36
6	Oleic acid	80	80337	21.94
7	Ledene oxide	93	93799	22.54

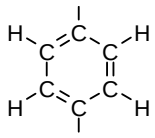
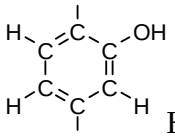
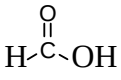
Table A-3 major components of the black pepper oil

No.	Component	pk	Peak code	Compositions with area%
1	1,2,3-propanetriol,glycerol,glyrol&glycerin, Osmoglyn	81	81999	0.91
2	Caparratriene,1,2,4-trimethyl cyclohexane	80	80579	0.20
3	Longifolen aldehyde	84	84231	2.21
4	4H-pyran-4-one,3-hydroxy-2-methyl,maltol	71	71256	0.19
5	2-ethyl-4-methyl-5,6-dihydro-2H-py	69	69255	0.19
6	Guaidiol	67	67214	0.22

Appendix B: FTIR Correlations Tables

Functional groups with absorbance, transmittance and frequency relation function with the type of vibration tables

Functional Group Names & Example compounds	**Absorption Ranges(cm⁻¹)** [Look for a single absorption in these regions, unless stated otherwise.]	**Type of Vibration causing IR absorption**
Alkanes:  Ethane	**3000-2800** (Note: The absorptions can be seen as several distinct peaks in this region.)	H-C-H Asymmetric & Symmetric Stretch
	1500-1440	H-C-H Bend
Alkenes:  1-Propene	**3100-3000**	C=C-H Asymmetric Stretch
	1675-1600	C-C=C Symmetric Stretch
Alkynes:  Propyne	**3300-3200**	≡C H Stretch
	2200-2100	C≡C Stretch
Aromatic Rings: Benzene	**3100-3000**	C=C-H Asymmetric Stretch
	1600-1580	C-C=C Symmetric

 H H		Stretch
	1500-1450	C-C=C Asymmetric Stretch
Phenols & Alcohols:  H Phenol  H HOH Methanol (Alcohol)	**3600-3100** (Note: Phenols MUST have Aromatic Ring Absorptions too.)	Hydrogen-bonded O-H Stretch (This peak usually appears much broader than the other IR absorptions.
Carboxylic Acids:  formic Acid	**3400-2400** *(This peak always covers the entire region with a **VERY BROAD** peak.)*	Hydrogen-bonded O-H Stretch *[Note: This peak can obscure other peaks in this region.]*
	1730-1650	C=O Stretch

Source: http://en.wikipedia.org/wiki/infrared spectroscopy correlations tables

Appendix C: Design Expert Model Equations

Total sum of squares, $SST = \sum_{i=1}^{n} \sum_{i=1}^{n} X_i - \bar{X}^2$

SST = SS $_{\text{Treatments}}$ + *SSE*, sum of squares due to treatments+ *sum of squares due to errors*

$SSE = \sum_{i=1}^{n} X_i - \bar{X}^2$ Where X= sample variables.

$MStreatment = \frac{SStreatment}{a-1}$, a-1 means degree of freedom, a=no of sample freedom. MS=mean squares.

A rough check for outliers made by standardized residuals**.**

$Dij = \frac{eij}{MSE}, MSE = \frac{SSE}{N-a}$ N=no of samples. Eij =error of percent or confidence interval.

The Analysis of Variance Table for the Single-Factor, Fixed Effects Model

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F_0
Between treatments	$SS_{\text{Treatments}} = n\sum_{i=1}^{a}(\bar{y}_{i.} - \bar{y}_{..})^2$	$a-1$	$MS_{\text{Treatments}}$	$F_0 = \frac{MS_{\text{Treatments}}}{MS_E}$
Error (within treatments)	$SS_E = SS_T - SS_{\text{Treatments}}$	$N-a$	MS_E	
Total	$SS_T = \sum_{i=1}^{a}\sum_{j=1}^{n}(y_{ij} - \bar{y}_{..})^2$	$N-1$		

Test statistics, $Fo = \frac{SStreatment/(a-1)}{SSE/(N-a)} = \frac{MStreatment}{MSE}$

The analysis of variance summary at the top of the computer output contains the usual sums of squares, degrees of freedom, mean squares, and test statistic *FO*. The column' 'Prob > F" is the P-value (actually, the upper bound on the P-value, because probabilities less than 0.0001 are defaulted to 0.0001).In addition to the basic analysis of variance, the program displays some additional useful information. The quantity "R-squared"

$R^2 = \frac{SS\,model}{SStotal}$

Table C-1 Diagnostic Case Statistics

Standard Order	Actual Value	Predictd Value	Residual	Leverage	Student Residual	Cook's Distance	Outlier t	Run Order
1	1.67	1.72	-0.046	0.497	-0.203	0.004	-0.199	1
2	2.41	2.68	-0.28	0.340	-1.052	0.057	-1.055	25
3	1.80	1.40	0.40	0.497	1.747	0.302	1.840	29
4	3.21	2.93	0.28	0.340	1.088	0.061	1.093	14
5	3.72	3.79	-0.078	0.211	-0.274	0.002	-0.269	17
6	2.15	2.42	-0.27	0.340	-1.017	0.053	-1.018	27
7	3.17	3.11	0.064	0.497	0.282	0.008	0.276	26
8	3.70	3.88	-0.18	0.340	-0.681	0.024	-0.672	8
9	2.50	2.40	0.097	0.497	0.423	0.018	0.415	13
10	2.28	2.30	-0.018	0.340	-0.070	0.000	-0.068	12
11	2.80	3.29	-0.49	0.211	-1.716	0.079	-1.802	16
12	2.27	2.04	0.23	0.340	0.893	0.041	0.889	30
13	3.25	3.41	-0.16	0.211	-0.545	0.008	-0.537	20
14	4.70	4.30	0.40	0.113	1.312	0.022	1.335	6
15	2.33	2.95	-0.62	0.211	-2.169	0.126	-2.390	4
16	3.36	3.49	-0.13	0.340	-0.479	0.012	-0.470	7
17	4.09	4.28	-0.19	0.211	-0.680	0.012	-0.672	9
18	3.07	2.84	0.23	0.340	0.892	0.041	0.888	2
19	2.70	2.50	0.20	0.497	0.888	0.078	0.884	19
20	3.35	3.52	-0.17	0.340	-0.636	0.021	-0.627	32
21	2.45	2.29	0.16	0.497	0.708	0.050	0.700	18
22	3.30	3.50	-0.20	0.340	-0.778	0.031	-0.771	28
23	4.83	4.43	0.40	0.211	1.411	0.053	1.446	22
24	2.60	3.10	-0.50	0.340	-1.916	0.189	-2.051	31
25	3.48	3.48	-2.010E-003	0.497	-0.009	0.000	-0.009	3
26	4.15	4.31	-0.16	0.340	-0.603	0.019	-0.594	24
27	3.15	2.89	0.26	0.497	1.154	0.132	1.163	11
28	4.67	4.30	0.37	0.113	1.213	0.019	1.227	5
29	4.30	4.30	-1.952E-003	0.113	-0.006	0.000	-0.006	15
30	4.45	4.30	0.15	0.113	0.488	0.003	0.479	10
31	4.27	4.30	-0.032	0.113	-0.105	0.000	-0.103	23
32	4.56	4.30	0.26	0.113	0.850	0.009	0.845	21

Appendix D: Experimental Result

Moisture content of seed, ((4kg-3.86kg)/4kg)*100%=3.5%,

The moisture content of leave was as follows: ((3.5kg-3.246kg)/3.5kg)*100%=7.257%.

Ash content of both materials:

Seed ash content= (100-97/100)*100=3%

Yield from different process conditions

Size		Temperature	Amount in ml	Amount in gm	Time	Yield%
750	250gm for each run	105	9.52	6	3:30	2.407
			11.5	7.244	4:00	2.897
				7.975	4:30	3.19

A, 105^{o}c, 11.5ml=7.244gm, yield = (7.244gm/250)*100%=2.897%

B, 127.5^{o}c, 14.75ml=9.29gm, yield= (9.29/250)*100%=3.716%

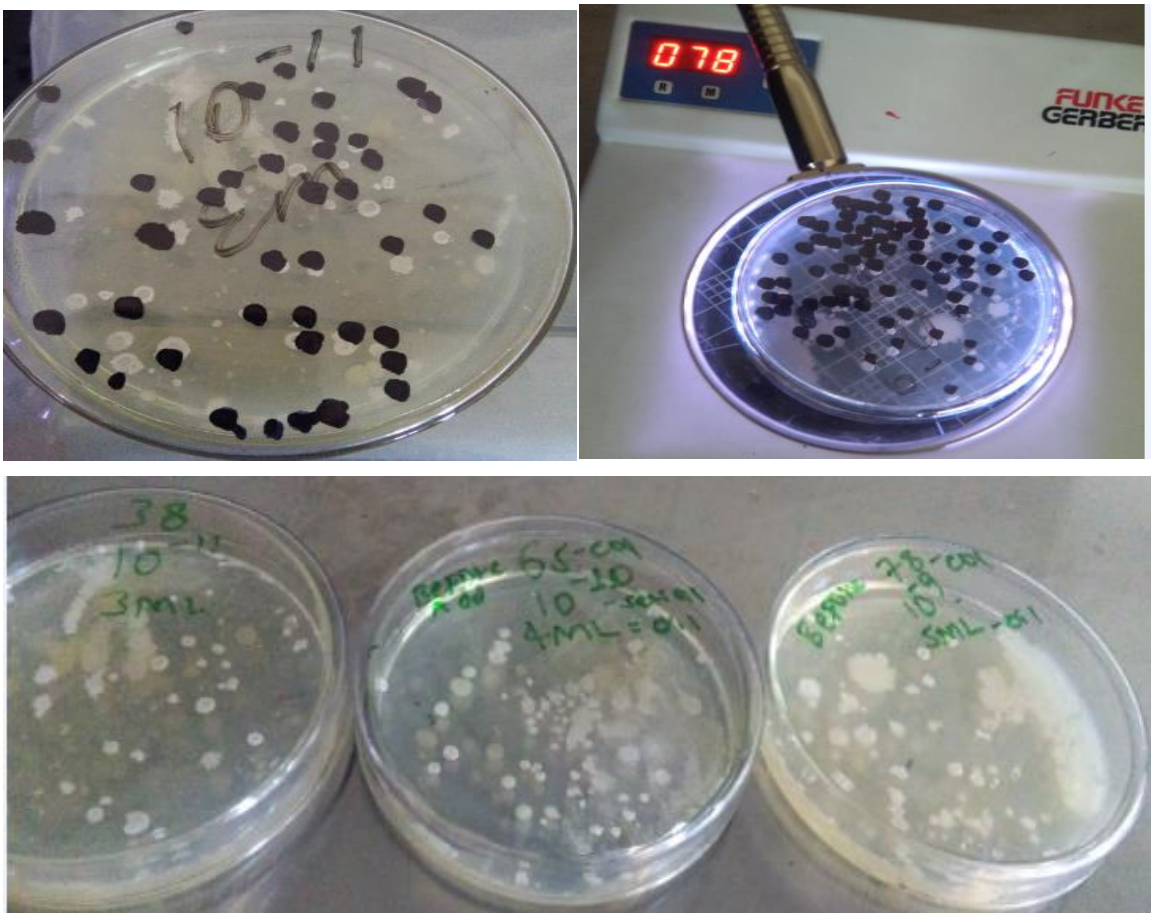
C, 150^{o}c, 11.75ml=7.4gm, yield= (7.4/250)*100%=2.96%

Appendix E: Laboratory Equipments, Materials and Photos

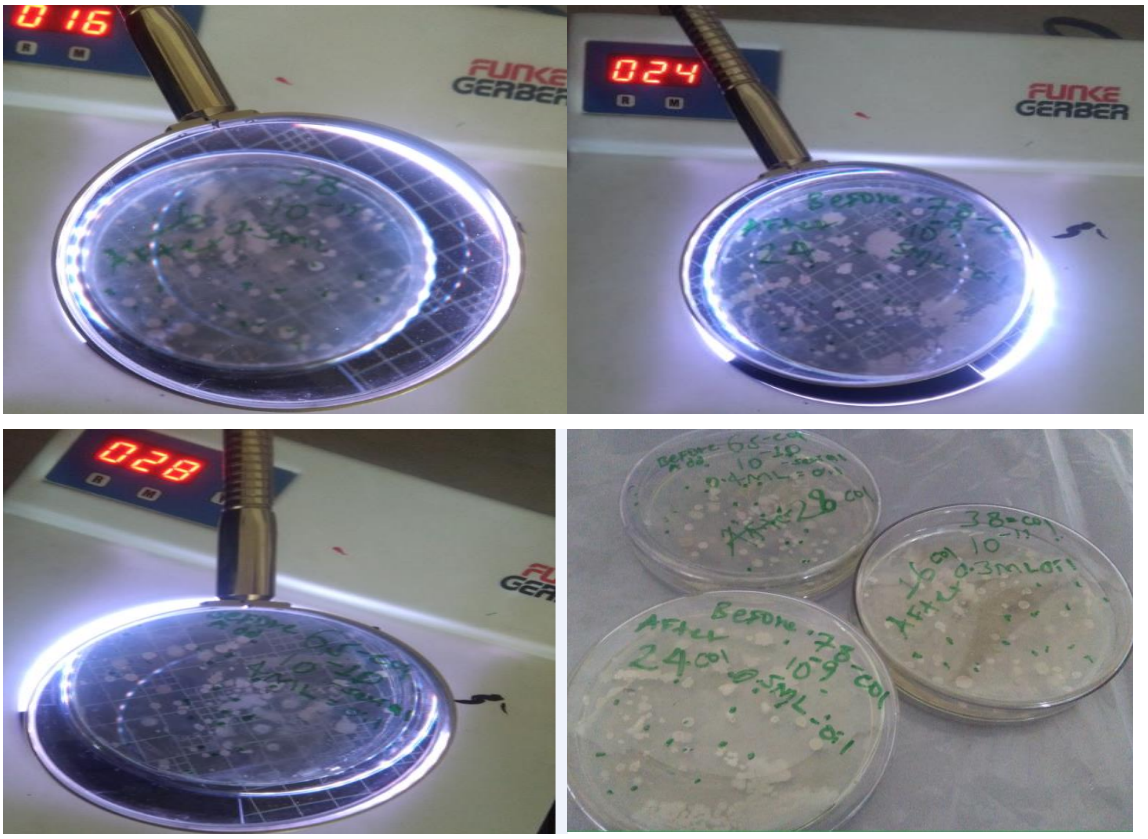
E1: Raw materials preparation and sample preparation with different size.

E2: Condenser and distiller

E3: During extraction of the oil and separation of oil by decanter



E4: Before addition of the oil the bacterial no of colonies.



E5: Bacterial number of colonies after addition of the oil.

E6: FTIR instruments

E7:GC/MS Instruments.